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(54) **Hog cholera virus vaccine and diagnostic**

Impfstoff und Diagnostikum für den Schweine-Cholera-Virus

Vaccin et test de diagnostic pour le virus du choléra porcin

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(73) Proprietor: **Akzo Nobel N.V.**
6824 BM Arnhem (NL)

(72) Inventors:
• **Meyers, Gregor**
D-7000 Stuttgart 80 (DE)
• **Rümenapf, Tillmann**
D-7400 Tübingen (DE)
• **Thiel, Heinz-Jürgen**
D-7400 Tübingen (DE)

(74) Representative: **Hermans, Franciscus G.M. et al**
Patent Department
AKZO NOBEL N.V.
Pharma Division
P.O. Box 20
5340 BH Oss (NL)

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Description

[0001] The present invention is concerned with a nucleic acid sequence, a recombinant nucleic acid molecule comprising such a nucleic acid sequence, a recombinant expression system comprising such a recombinant nucleic acid molecule, a polypeptide characteristic of the hog cholera virus, a vaccine comprising such a polypeptide or recombinant expression system as well as a method for the preparation of such vaccines.

[0002] Classical swine fever or hog cholera (HC) represents an economically important disease of swine in many countries worldwide. Under natural conditions, the pig is the only animal known to be susceptible to HC. Hog cholera is a highly contagious disease which causes degeneration in the walls of capillaries, resulting in hemorrhages and necrosis of the internal organs. In the first instance hog cholera is characterized by fever, anorexia, vomiting and diarrhea which can be followed by a chronic course of the disease characterized by infertility, abortion and weak offsprings of sows. However, nearly all pigs die within 2 weeks after the first symptoms appear.

[0003] The causative agent, the hog cholera virus (HCV) has been shown to be structurally and serologically related to bovine viral diarrhoea virus (BVDV) of cattle and to border disease virus (BDV) of sheep. These viruses are grouped together into the genus pestivirus within the family togaviridae. The nature of the genetic material of pestiviruses has long been known to be RNA, i.e. positive-strand RNA which lacks significant polyadenylation. The HCV probably comprises 3-5 structural proteins of which two are possibly glycosylated. The number of non-structural viral proteins is unknown.

[0004] Modified HCV vaccines (comprising attenuated or killed viruses) for combating HC infection have been developed and are presently used. However, infection of tissue culture cells to obtain HCV material to be used in said modified virus vaccines, leads to low virus yields and the virions are hard to purify. Modified live virus vaccines always involve the risk of inoculating animals with partially attenuated pathogenic HCV which is still pathogenic and can cause disease in the inoculated animal or offspring and of contamination by other viruses in the vaccine. In addition the attenuated virus may revert to a virulent state.

[0005] There are also several disadvantages using inactivated vaccines, e.g. the risk of only partial inactivation of viruses, the problem that only a low level of immunity is achieved requiring additional immunizations and the problem that antigenic determinants are altered by the inactivation treatment leaving the inactivated virus less immunogenic.

[0006] Furthermore, the usage of modified HCV vaccines is not suited for eradication programmes.

[0007] Until now, according to our knowledge diagnostic tests in swine which can distinguish between HCV or BVDV infection are not available. This is important as BVDV infection in pigs is of lower significance than HCV infection which means that BVDV infected pigs do not have to be eradicated.

[0008] Vaccines containing only the necessary and relevant HCV immunogenic material which is capable of eliciting an immune response against the pathogen do not display abovementioned disadvantages of modified vaccines.

[0009] According to the present invention a nucleic acid sequence which is a fragment of the hog cholera virus genome, and encoding a polypeptide characteristic of hog cholera virus which polypeptide comprises the amino acid sequence located within amino acid position 689-1067 has been found. Both the nucleic acid sequence and the polypeptide can be used for the preparation of a vaccine containing only the necessary and relevant immunogenic material for immunizing animals against HCV infection. "Nucleic acid sequence" refers both to a ribonucleic acid sequence and a deoxy-ribonucleic acid sequence.

[0010] A nucleic acid sequence according to the present invention is shown in figure 2. As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in an other codon but still coding for the same amino acid, e.g. the codon for the amino acid glutamic acid is both GAT and GAA. Consequently, it is clear that for the expression of a polypeptide with the amino acid sequence shown in figure 2 encoded by a nucleic acid sequence according to the invention, use can be made of a nucleic acid sequence with such an alternative codon composition different from the nucleic acid sequence shown in figure 2.

[0011] Also included within the scope of the invention are nucleic acid sequences which hybridize under stringent conditions to the nucleic acid sequence shown in figure 2 encoding the polypeptide comprising the amino acid sequence located within amino acid position 689-1067. These nucleic acid sequences are related to the nucleic acid sequence shown in figure 2 but may comprise nucleotide substitutions, mutations, insertions, deletions etc. and encode polypeptides which are functionally equivalent to the polypeptide defined above shown in figure 2, i.e. the amino acid sequence of a related polypeptide is not identical with the amino acid sequence shown in figure 2 but features corresponding immunological properties characteristic for HCV.

[0012] The nucleic acid sequence shown in figure 2 is a cDNA sequence derived from the genomic RNA of HCV. This continuous sequence is 12284 nucleotides in length, and contains one long open reading frame (ORF), starting with the ATG codon at position 364 to 366 and ending with a TGA codon as a translational stop codon at position 12058 to 12060. This ORF consists of 3898 codons capable of encoding 435 kDa of protein.

[0013] In vivo, during HCV replication in an infected cell, this protein is synthesized as a polyprotein precursor molecule which is subsequently processed to fragment polypeptides by (enzymatic) cleavage of the precursor molecule.

These fragments form after possible post-translational modifications the structural and non-structural proteins of the virus. The nucleic acid sequence according to the present invention contains the genetic information for such a fragment with immunizing properties against HCV or immunological properties characteristic for HCV or contains the genetic information for a portion of such a fragment which still has the immunizing properties or the immunological properties characteristic for HCV.

[0014] Fragment polypeptides of the polypeptide according to figure 2 and the portions thereof, which can be used for the immunisation of animals against HC or for diagnosis of HC are located within the amino acid position about 1-249, 263-487, 488-688 or 689-1067.

[0015] The 1-249 region essentially represents the core protein whereas the 263-487, 488-688 and 689-1067 regions essentially represent glycoproteins of 33 kD, 44/48 kD and 55 kD respectively. Within the scope of the invention is a nucleic acid sequence comprising the genetic information for at least the 55 kD coding region mentioned above or portions thereof.

[0016] A preferred region to be incorporated into a vaccine against HCV infection is the region corresponding to the 55 kD protein of HCV or a portion thereof still having immunizing activity.

[0017] Furthermore, a nucleic acid sequence at least comprising the coding sequences for said 55 kD protein or portion thereof can advantageously be applied according to the present invention.

[0018] In addition, a preferred portion of the HCV 55 kD protein, which can be used for immunization of pigs against HCV infection, is determined by analyses of HCV deletion mutants with anti-55 kD protein monoclonal antibodies having virus neutralizing activity. Such a portion comprising an epitope spans the amino acid sequence about 812-859 and is coded by the nucleotide sequence about 2799-2938. A nucleic acid sequence at least comprising said nucleotide sequence forms part of the present invention too.

[0019] A nucleic acid sequence according to the invention which can be used for the diagnosis of HCV infection in pigs and which can be applied to discriminate HCV from BVDV can be derived from the gene encoding the 55 kD protein.

[0020] Preferably, such a nucleic acid sequence is derived from the nucleotide sequences 2587-2619 or 2842-2880, both sequences being part of the gene encoding the 55 kD protein. A preferred oligonucleotide for diagnostic purposes is:

5' - CCT ACT AAC CAC GTT AAG TGC TGT GAC TTT AAA - 3'

OR

5' - TTC TGT TCT CAA GGT TGT GGG GCT CAC TGC TGT GCA CTC
- 3'

[0021] Moreover, a nucleic acid sequence comprising at least a sub-sequence of said oligonucleotides and which still can be used to differentiate between HCV and BVDV forms part of the invention.

[0022] The invention also relates to a test kit to be used in an assay, this test kit containing a nucleic acid sequence according to the invention.

[0023] Preferably the test kit comprises an oligonucleotide mentioned above or a nucleic acid sequence comprising at least a sub-sequence thereof.

[0024] Variations or modifications in the polypeptide shown in figure 2 or fragments thereof, such as natural variations between different strains or other derivatives, are possible while retaining the same immunologic properties. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said polypeptide.

[0025] Moreover, the potential exists, in the use of recombinant DNA technology, for the preparation of various derivatives of the polypeptide shown in figure 2 or fragments thereof, variously modified by resultant single or multiple amino acid substitutions, deletions, additions or replacements, for example by means of site directed mutagenesis of the underlying DNA. All nucleic acid sequences encoding such modifications resulting in derivatives of the polypeptide defined above shown in figure 2 or fragments thereof are included within the scope of the present invention so long as the essential characteristic activity of said polypeptide or fragment thereof, remains unaffected in essence.

[0026] RNA isolated from pelleted virions was isolated and used for the synthesis of cDNA. This cDNA was cloned in phage λ gt11 and the respective library was amplified and screened with goat anti-HCV antiserum. Two positive clones could be identified and shown to have inserts with sizes of 0,8 kb and 1,8 kb. The 0,8 kb λ gt11 insert was partially sequenced (see figure 3) and determined to be located between about 1,2 and 2,0 kb on the HCV genome (see figure 2).

[0027] The invention also relates to a test kit to be used in an assay, this test kit containing a nucleic acid sequence according to the invention.

[0028] A nucleic acid sequence according to the present invention can be ligated to various vector nucleic acid

molecules such as plasmid DNA, bacteriophage DNA or viral DNA to form a recombinant nucleic acid molecule. The vector nucleic acid molecules preferably contain DNA sequences to initiate, control and terminate transcription and translation. A recombinant expression system comprising a host containing such a recombinant nucleic acid molecule can be used to allow for a nucleic acid sequence according to the present invention to express a polypeptide encoded by said nucleic acid sequence. The host of above-mentioned recombinant expression system can be of prokaryotic origin, e.g. bacteria such as *E. coli*, *B. subtilis* and *Pseudomonas*, viruses such as vaccinia and fowl pox virus or eucaryotic origin such as yeasts or higher eucaryotic cells such as insect, plant or animal cells.

[0029] Immunization of animals against HCV can, for example, be achieved by administering to the animal a polypeptide encoded by a nucleic acid sequence according to the invention as a so-called "sub-unit" vaccine. The subunit vaccine according to the invention comprises a polypeptide generally in a pure form, optionally in the presence of a pharmaceutically acceptable carrier.

[0030] Small fragments are preferably conjugated to carrier molecules in order to raise their immunogenicity. Suitable carriers for this purpose are macromolecules, such as natural polymers (proteins, like key hole limpet hemocyanin, albumin, toxins), synthetic polymers like polyamino acids (polylysine, polyalanine), or micelles of amphiphilic compounds like saponins. Alternatively these fragments may be provided as polymers thereof, preferably linear polymers. Polypeptides to be used in such subunit vaccines can be prepared by methods known in the art, e.g. by isolation said polypeptides from hog cholera virus, by recombinant DNA techniques or by chemical synthesis.

[0031] If required the polypeptides encoded by a nucleic acid sequence according to the invention to be used in a vaccine can be modified in vitro or in vivo, for example by glycosylation, amidation, carboxylation or phosphorylation.

[0032] An alternative to subunit vaccines are "vector" vaccines. A nucleic acid sequence according to the invention is integrated by recombinant techniques into the genetic material of another micro-organism (e.g. virus or bacterium) thereby enabling the micro-organism to express a polypeptide according to the invention. This recombinant expression system is administered to the animal to be immunized whereafter it replicates in the inoculated animal and expresses the polypeptide resulting in the stimulation of the immune system of the animal. Suitable examples of vaccine vectors are pox viruses (such as vaccinia, cow pox, rabbit pox), avian pox viruses (such as fowl pox virus) pseudorabies virus, adeno viruses, influenza viruses, bacteriophages or bacteria (such as *Escherichia coli* and *Salmonella*).

[0033] The recombinant expression system having a nucleic acid sequence according to the invention inserted in its nucleic acid sequence can for example be grown in a cell culture and can if desired be harvested from the infected cells and formed to a vaccine optionally in a lyophilized form. Said genetically manipulated micro-organism can also be harvested from live animals infected with said micro-organism. Abovementioned recombinant expression system can also be propagated in a cell culture expressing a polypeptide encoded by a nucleic acid sequence according to the invention, whereafter the polypeptide is isolated from the culture.

[0034] A vaccine comprising a polypeptide encoded by a nucleic acid sequence according to the invention or a recombinant expression system according to the present invention can be prepared by procedures well-known in the art for such vaccines. A vaccine according to the invention can consist inter alia of whole host, host extract, partially or completely purified polypeptide or a partially or completely purified recombinant expression system as above-mentioned.

[0035] The vaccine according to the invention can be administered in a conventional active immunization scheme: single or repeated administration in a manner compatible with the dosage formulation and in such amount as will be therapeutically effective and immunogenic. The administration of the vaccine can be done, e.g. intradermally, subcutaneously, intramuscularly, intra-venously or intranasally. For parenteral administration the vaccines may additionally contain a suitable carrier, e.g. water, saline or buffer solution with or without adjuvants, stabilizers, solubilizers, emulsifiers etc.

[0036] The vaccine may additionally contain immunogens related to other diseases or nucleic acid sequences encoding these immunogens like antigens of parvovirus, pseudorabies virus, swine influenza virus, TGE virus, rotavirus, *Escherichia coli*, *Bordetella*, *Pasteurella*, *Erysipelas* etc. to produce a multivalent vaccine.

[0037] Polypeptides encoded by a nucleic acid sequence according to the present invention can also be used in diagnostic methods to detect the presence of HCV antigen or antibody in an animal. Moreover, nucleic acid sequences according to the invention can be used to produce polypeptides to be used in above-mentioned diagnostic methods or as a hybridisation probe for the detection of the presence of HCV nucleic acid in a sample.

Example 1

Immunological identification of cDNA clones

[0038] Infection of cells and harvesting of virus. PK15 and 38A₁D cells were grown in DMEM with 10% FCS and were infected in suspension by the virulent HCV strain Alfort in a volume of 20-30 ml at a cell concentration of 5×10^7 /ml at 37 °C for 90 min with an m.o.i. of 0.01 to 0.001 (as determined by immunofluorescence assay). Thereafter, the

PK15 cells were seeded in tissue culture plates (150 mm diameter), while the suspension cells 38A₁D were incubated in bottles with gentle stirring (Tecnomara, Switzerland). For cDNA synthesis, the tissue culture supernatant was harvested 48 hours after infection, clarified at 12,000 g, and afterwards the virus pelleted in a TFA 20 rotor (Contron, Italy) at 54,000 g for 12 hours.

Example 1

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[0040] Preparation of goat anti-HCV serum. A fibroblastic cell strain was established from the skin biopsy of a young goat by standard cell culture techniques. The cells were initially grown in F-10 medium with 10% FCS and later in DMEM with 10% FCS. Goat fibroblasts were infected with HCV. Over the first 26 hours p.i., the cells were washed every 8 hours 3 times with PBS and afterwards incubated in DMEM with 10% preimmune goat serum (PGS). 48 hours p.i., the tissue culture supernatant was harvested and used as stock virus. Before immunization, goat cells for 30 tissue culture dishes (150 mm diameter) were kept for 3 passages in medium with 10% PGS and then infected with the stock virus. 48 hours p.i., the goat was immunized with X-ray-inactivated pelleted virus and infected cells. Both were emulsified in Freund's adjuvant (complete for basis immunization, incomplete for booster injections) and injected subcutaneously. To obtain antibodies recognizing denatured molecules, the antigen preparations were incubated in 0.2% SDS, 3 mM DTT at 95 °C for 5 min before injection.

[0041] RNA preparation, cDNA synthesis and cloning. RNA from virions was isolated by using the guanidine thiocyanate method described by Chirgwin et al. (1979). RNA from pelleted virions (5 µg total RNA, approximately 0.5 µg HCV RNA) and 0.1 µg of random hexanucleotide primer (Pharmacia, Sweden) in 20 µl of water were heated to 65 °C for 10 min, chilled on ice, and adjusted to first strand buffer (50 mM Tris-HCl pH 8.3; 30 mM KCl; 8 mM MgCl₂; 1 mM DTT, dATP, dCTP, dGTP, dTTP 1 mM each and 500 units RNAGuard [Pharmacia, Sweden] per ml) in a final volume of 32 µl. 35 units of AMV reverse transcriptase (Life Sciences Inc., USA) were added. After 1 hour at 43 °C the reaction mixture was added to one vial of second strand synthesis mixture (cDNA synthesis kit, Pharmacia, Sweden). Second strand synthesis, preparation of blunt ends, and Eco RI adaptor ligation and phosphorylation were done as recommended by the supplier.

[0042] The cDNA was size-fractionated by preparative agarose gel electrophoresis. The part of the gel containing DNA molecules smaller than 0.5 kb was discarded. The remaining DNA was concentrated by running the gel reversely for 15 min and extracted from the agarose after 3 cycles of freezing and thawing with phenol.

[0043] Ethanol co-precipitated cDNA and λgt11 DNA (1 µg EcoRI digested dephosphorylated arms, Promega, USA) was ligated by 3 units of T4 DNA ligase (Pharmacia, Sweden) in a total volume of 10 µl ligase buffer (30 mM Tris-HCl pH 7.4; 10 mM MgCl₂; 10 mM DTT; 1 mM ATP). In vitro packaging with a commercially available extract (Packagene, Promega, USA) and infection of E.coli K12 cells, strain Y 1090, with resulting phages was performed as recommended by the supplier. The library was amplified once as described (Davis et al., 1986).

[0044] Screening of λgt11 library. Screening was basically performed as described (Young and Davis, 1983) using the Protoblot system purchased from Promega, USA (Huynh et al., 1985) and a serum dilution of 10^{-3} . For background reduction the goat anti HCV serum was treated with E.coli lysate (strain Y1090) at 0.8 mg/ml (Huynh et al., 1985). Two positive clones having inserts of 0.8 kb and 1.8 kb, respectively could be identified.

[0045] Nick translation and Northern hybridization. 50 ng of the 0.8 kb HCV nucleic acid sequence labeled with [α^{32} P] dCTP (3000 Ci per mMole, Amersham Buchler, FRG) by nick translation (nick translation kit, Amersham Buchler, FRG) was hybridized to Northern filters at a concentration of 5 ng per ml of hybridization mixture (5 x SSC; 1 x Denhardt's; 20 mM sodium phosphate pH 6.8; 0.1% SDS and 100 µg yeast tRNA [Boehringer-Mannheim, FRG] per ml) at 68 °C for 12 to 14 hours. Membranes were then washed as described (Keil et al., 1984) and exposed at -70 °C to Kodak X-Omat AR films for varying times using Agfa Curix MR 800 intensifying screens.

[0046] The 0.8 kb nucleic acid sequence hybridized not only to intact HCV RNA but also to degradation products thereof. The 0.8 kb nucleic acid sequence did not hybridize to the 1.8 kb nucleic acid sequence, indicating that these two nucleic acid sequences correspond with fragments of the HCV genome which are not located in the same region of the genomic RNA.

[0047] Nucleotide sequencing. Subcloning of HCV specific phage DNA inserts into plasmid pEMBL 18 plus was done

according to standard procedures (Maniatis et al., 1982). Single-stranded DNA of recombinant pEMBL plasmids was prepared as described (Dente et al., 1985). Dideoxy sequencing reactions (Sanger et al., 1977) were carried out as recommended by the supplier (Pharmacia, Sweden).

Example 2

Molecular cloning and nucleotide sequence of the genome of HCV

[0048] RNA preparation, cDNA synthesis and cloning. RNA preparation, cDNA synthesis, size selection and ligation of co-precipitated cDNA and λ gt10 DNA (1 μ g EcoRI digested dephosphorylated arms, Promega, USA) were done as described above. In vitro packaging of phage DNA using Packagene (Promega, USA) and titration of phages on E.coli strain C 600 HFL were performed as suggested by the supplier. The library was amplified once (Davis et al., 1986), and replicas transferred to nitrocellulose membranes (Amersham Buchler, FRG) (Benton and Davis, 1977) were hybridized with oligonucleotides as described above for Northern hybridization. Screening with cDNA fragments labeled with [α^{32} P] dCTP by nick translation (nick translation kit, Amersham Buchler, FRG) was done as described by Benton and Davis (1977). Positive clones were plaque purified and inserts subcloned into pEMBL plasmids (Maniatis et al., 1982; Dente et al., 1985; Davis et al., 1986).

[0049] A 32 P 5'-end labeled oligonucleotide of 17 bases complementary to the RNA sequence encoding the amino acid sequence Cys Gly Asp Asp Gly Phe was used for screening a λ gt10 cDNA library. This oligonucleotide which hybridized to the about 12 kb genomic RNA of HCV, identified inter alia a clone with an insert of 0.75 kb, which hybridized also to HCV RNA. This 0.75 kb nucleic acid sequence which represents a fragment of the HCV genome together with the 0.8 kb λ gt11 nucleic acid sequence insert were used for further library screening resulting in a set of overlapping HCV nucleic acid sequences of which the relative positions and restriction site maps are shown in figure 1. These nucleic acid sequence fragments of the HCV genome are located between the following nucleic acid positions

4.0 kb fragment	27-4027
4.5 kb fragment	54-4494
0.8 kb fragment	1140-2002
4.2 kb fragment	3246-7252
5.5 kb fragment	6656-11819

and within about the following nucleic acid positions

3.0 kb fragment	8920-11920
1.9 kb fragment	10384-12284
0.75 kb fragment	10913-11663

[0050] Nucleotide sequencing. For complete nucleotide sequence determination exonuclease III and nuclease S1 (enzymes from Boehringer Mannheim, FRG) were used to establish deletion libraries of HCV derived cDNA inserts subcloned into pEMBL 18+ or 19+ plasmids (Hennikoff, 1987). Dideoxy sequencing (Sanger et al. 1977) of single stranded (Dente et al., 1985) or double stranded DNA templates was carried out using the T7 polymerase sequencing kit (Pharmacia, Sweden).

[0051] From the cDNA fragments a continuous sequence of 12284 nucleotides in length could be determined as shown in figure 2. This sequence contains one long open reading frame (ORF), starting with the ATG codon at position 364 to 366 and ending with TGA as a translational stop codon at 12058 to 12060. This ORF consists of 3898 codons capable of encoding a 435 kDa protein with an amino acid sequence shown in figure 2. Three nucleotide exchanges were detected as a result of differences in nucleotide sequence caused by possible heterogeneity of the virus population, two of which resulted in changes in the deduced amino acid sequence (figure 2).

[0052] It is concluded that almost the complete HCV genome has been cloned and sequenced by the procedures described above.

[0053] The 0.8 kb λ gt11 nucleic acid sequence encoding an immunogenic HCV polypeptide identified with anti HCV serum was partially sequenced (see figure 3) which revealed that this sequence is located within 1.2 and 2.0 kb on the HCV RNA.

Example 3Molecular cloning and expression of fusion proteins of HCV

- 5 [0054] cDNA fragments derived from two regions of the HCV genome, i.e. the 0,8 kb λ gt11 insert of example 1 encoding amino acids 262-546 (see figure 2) and the nucleic acid sequence encoding amino acids 747-1071 (figure 2), are expressed as fusion proteins in the pEx system (Strebel, K. et al., 1986).
- [0055] Bacterial extracts were separated by SDS-PAGE and stained according to standard procedures, and then tested for reactivity with the goat anti-HCV serum of example 1 in a Western blot.
- 10 [0056] The HCV specific fusion proteins were partially purified by SDS-PAGE and transferred to nitrocellulose and incubated with the goat anti-HCV serum. Specific antibodies against said fusion proteins were obtained after elution.
- [0057] Antibodies specific for the above-mentioned fusion proteins were employed in a radio-immuno precipitation assay.

15 Results

- [0058] Both fusion proteins expressed in the pEx system were clearly identified as HCV specific after reaction with the goat anti-HCV serum.
- [0059] Monospecific antiserum prepared against both fusions proteins precipitated HCV glycoproteins.
- 20 [0060] Antibodies specific for the 262-546-fusion protein precipitated the 44/48 kD and 33 kD protein, antibodies specific for the 747-1071-fusion protein precipitated the 55 kD protein from virus infected cells.

Example 425 Molecular cloning and expression of structural proteins via vaccinia virus

- [0061] A fragment of the 4,0 kb clone shown in figure 1 (pHCK11) is prepared starting at the *HinfI* restriction site (nucleotide 372) and ending at an artificial *EcoRI* site (nucleotide 4000) (Maniatis et al. 1982). For the 5' end an oligo-nucleotide adaptor was synthesized which contained an overhang compatible to *BamHI*, the original ATG(364-366) as
- 30 translational start codon and a protruding end compatible to *HinfI* at the 3' end.

35

	5'	GATCCACCA ATG GAGTT	<i>HinfI</i>
	<i>BamHI</i>	GTGGTACCTCAACTTA	5'

At the 3' end of the construct a translational stop codon was introduced by deletion of the *EcoRI* protruding end with Mung bean nuclease and ligation into a blunt-end *StuI/EcoRI* adaptor residue:

40

	5'	GCCTGAATTC	3' <i>EcoRI</i>
		CGGACTTAAG	

45 (Maniatis et al. 1982).

- [0062] Prior to inserting above-mentioned HCV sequences into vaccinia virus the heterologous gene is cloned into a recombination vector. For this purpose a pGS62 plasmid (Cranage, M.P. et al. 1986) was used which contains a cloning site downstream the P7.5K promoter within the 4.9kb thymidine kinase sequence. The cloning site comprises three unique restriction sites, *BamHI*, *SmaI* and *EcoRI*. The recombination vector pGS62-3.8 was established by ligation
- 50 of the described HCV sequence (372-4000) together with the adaptors into the *BamHI/EcoRI* digested pGS62.

- [0063] Based on the plasmid a set of 15 deletion mutants was established. By treatment with *ExonucleaseIII* (Henrikof et al., 1987) subsequent shortening of the HCV cDNA from the 3' end was performed. All deletions are located within the region coding for the HCV 55 kD protein by removal of about 100bp; most of the 55 kD protein is lost in mutant 15 ending at nucleotide 2589. *ExoIII* shortened cDNA clones were ligated into the pGS62 giving rise to
- 55 pGS62-3.8Exo 1-15 (figure 4).

[0064] CV1 cells were infected with vaccinia (strain Copenhagen, mutant TS7) at a MOI of 0.1. Three hours after infection pGS62-3.8 DNA as well as vaccinia WR DNA were transfected by the $\text{Ca}_3(\text{PO}_4)_2$ precipitation method and incubated for two days. Virus progeny was harvested and selected for tk-phenotype on 143 tk-cells in the presence of

brom-deoxy-Uridine (100 µg/ml). This selection was performed at least twice followed by two further cycles of plaque purification.

Characterization of vaccinia-HCV recombinants

[0065] CVI cells were infected at an MOI between 2 and 10 with vaccinia-HCV recombinants and incubated for 8-16 hours. After fixation of the cells indirect immunofluorescence was performed using either monoclonal antibodies specific for HCV 55 kD protein or polyvalent anti-HCV sera. In all cases a cytoplasmatic fluorescence could be demonstrated.

[0066] After radioimmunoprecipitation and western blot analysis of cells infected with vaccinia recombinants four HCV-specific proteins were detected. By labeling with [³H] glucosamine it was shown that three of these proteins are glycosylated. The apparent molecular weights of these proteins were identical to those found in HCV infected cells with HCV specific sera, namely 20 kD(core), 44/48 kD, 33 kD and 55 kD.

[0067] Proteolytic processing and modifications appear to be authentic since HCV proteins produced by expression via vaccinia virus have the same apparent molecular weights as in HCV infected cells.

Induction of neutralizing antibodies against HCV in mice.

[0068] Four groups of mice (3 mice/group) were infected once with

a.	Vaccinia WR wildtype	(5x10 ⁶ pfu/individual)	WR
b.	Vaccinia 3.8 recombinant	(5x10 ⁷ pfu/individual)	VAC3.8
c.	Vaccinia 3.8Exo 4 (55 kD deleted)	(5x10 ⁷ pfu/individual)	VAC3.8Exo 4
d.	Vaccinia 3.8Exo 5	(5x10 ⁷ pfu/individual)	VAC3.8Exo 5
e.	Vaccinia 3.8Exo 15 (55 kD deleted)	(5x10 ⁷ pfu/individual)	VAC3.8Exo 15

by injection of purified virus intraperitoneally.

Mice were bled three weeks later. The reactivity of the sera was checked in a virus neutralization assay with HCV (Alfort) on PK[15] cells after serial dilution. (Römenapf, T. et al. 1989).

Neutralization titers		
a.	WR	<1:2
b.	VAC3.8	1:96
c.	VAC3.8Exo 4	1:96
d.	VAC3.8Exo 5	<1:2
e.	VAC3.8Exo 15	<1:2

[0069] From the above it can be concluded that vaccinia virus containing a nucleic acid sequence comprising the genetic information for all structural proteins (VAC3.8) is able to induce virus neutralizing antibodies in mice, while incomplete constructs VAC3.8Exo 5-15 and WR are not.

[0070] As all deletions are located within the region coding for HCV 55 kD protein (most of the 55 kD protein is lost in mutant 15 ending at nucleotide 2589) and the other structural proteins are still being expressed by the recombinant vaccinia virus, it is clear that the 55 kD protein is responsible for the induction of HCV neutralizing antibodies.

Example 5

Immunization of pigs with VAC3.8

[0071] Out of three piglets (about 20 kg in weight) one animal (no. 28) was infected with wild type vaccinia virus (WR strain) and the other two (no. 26, 27) with recombinant VAC3.8 (i.p., i.v. and i.d., respectively). For infection 1x10⁶ pfu of vaccinia virus is applied to each animal.

[0072] Clinical signs in the course of vaccinia infection were apparent as erythema at the side of scarification and fever (41 °C) at day six after infection.

Titers against vaccinia and hog cholera virus:

[0073] Three weeks after infection the reactivity of the respective sera against vaccinia (WR on CVI cells) and HCV (Alfort on PK15 cells) was checked.

[0074] Neutralization was assayed after serial dilution of the sera by checking for complete absence of cpe (vaccinia)

or specific signals in immunofluorescence (HCV). (Rümenapf, T. et al. 1989).

Neutralization titers against vaccinia:	
pig 28 (WR)	1:8
pig 26 (VAC3.8)	1:16
pig 27 (VAC3.8)	1:16

Neutralization titers against HCV:	
pig 28 (WR)	<1:2
pig 26 (VAC3.8)	1:32
pig 27 (VAC3.8)	1:16

Challenge with HCV:

[0075] Four weeks after immunization with vaccinia each of the pigs was challenged by infection with 5×10^7 TCID₅₀ HCV Alfort. Virus was applicated oronasal according to the natural route of infection. This amount of virus has been experimentally determined to be compulsory lethal for pigs.

[0076] On day five after the challenge infection pig 28 revealed fever of 41.5 °C and kept this temperature until day 12. The moribund animal was killed that day expressing typical clinical signs of acute hog cholera.

[0077] Both pigs (26, 27) immunized with VAC3.8 did not show any sign of illness after the challenge with HCV for more than 14 days.

Example 6

Construction of a 55 kD protein expression vector

[0078] Clone pHCK11 is digested with restriction enzymes SacI and HpaI according to standard techniques. The resulting 1.3 kb fragment, located between nucleotides 2672 (AGCTC) and 3971 (GTT) comprising most of HCV 55 kD protein, is isolated and cloned into the pseudorabies virus (PRV) gX gene (Maniatis et al. 1982).

[0079] Briefly, the cloned gX sequence was digested with SacI and ApaI. The ApaI 5' protruding ends were made blunt by filling up with Klenow fragment. After ligation the putative gX leader peptide coding sequence was located just upstream of the inserted HCV 55 kD sequence.

[0080] A translational stop codon downstream the HCV sequence was introduced by digestion with Bgl II (Bgl II site: 3936-3941) and religation after filling up the overhangs with Klenow fragment. This construct was placed downstream of the PRV gX promoter (clone 16/4-1.3). Clone 16/4-1.3 was transfected into MDBK cells by the DEAE dextran method (Maniatis et al. 1989). 16 h. later cells were infected with PRV (m.o.i. = 1). 4 h. post infection cells were fixed with a mixture of cold (-20 °C) methanol/acetone. Indirect immunofluorescence with monoclonal antibodies (MABs) anti-HCV 55 kD protein revealed a specific signal in 5-10% of the cells. PRV infected cells without transfection and cells only transfected with clone 16/4-1.3 did not show any signal in this assay.

Brief description of the drawings

[0081] Fig. 1 displays physical maps of different HCV derived cDNA clones and their position relative to the RNA genome (upper line). Two HCV derived cDNA clones isolated after screening with either the antibody probe (0.8 kb clone) or the degenerated oligonucleotide probe (0.75 kb clone) are shown in the second line. The cDNA fragments chosen for nucleotide sequencing are indicated below. All numbers represent sizes of DNA fragments in kb. Restriction sites: B = Bgl II; E = EcoRI; H = Hind III; K = Kpn I; S = Sal I; Sm = Sma I.

[0082] Fig. 2 depicts a nucleic acid sequence of HCV and deduced amino acid sequence of the long open reading frame. Nucleotide exchanges between different cDNA clones and resulting changes in amino acid sequence are indicated. The part of the sequence corresponding to the oligonucleotide used for screening is underlined.

[0083] Fig. 3 shows the cDNA sequence from part of the 0.8 kb HCV insert of a λ gt11 clone and the deduced amino acid sequence in one-letter code.

[0084] Fig. 4 shows the length of the HCV DNA cloned in the pGS62 vector. A set of 15 deletion mutants derived from cDNA clone pHCK11 was established by treatment with Exonuclease III and cloned in the pGS62 vector giving rise to pGS62-3.8Exo 1-15. 3' end nucleotides are indicated.

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30 Claims

Claims for the following Contracting States : BE, CH, DK, FR, GB, IT, LI, NL, SE

- 35 1. A nucleic acid sequence corresponding to a fragment of the hog cholera virus genome, and encoding a hog cholera virus polypeptide or an antigenic fragment thereof, capable of inducing virus neutralising antibodies, which polypeptide comprises the amino acid sequence located within the amino acid position 689-1067 shown in Fig 2 or is a functional equivalent thereof.
- 40 2. A nucleic acid sequence according to claim 1 characterised in that it comprises either the nucleotide sequence 2428-3564 shown in Fig 2 or a fragment thereof, or a nucleotide sequence which hybridises under stringent conditions to said nucleotide sequence shown in Fig 2.
- 45 3. A recombinant nucleic acid molecule comprising a vector nucleic acid molecule and a nucleic acid sequence according to claim 1 or claim 2.
4. A host cell capable of producing a polypeptide of hog cholera virus comprising a recombinant nucleic acid molecule according to claim 3.
- 50 5. A host cell according to claim 4 characterised in that the host cell is a bacterium.
6. A recombinant virus containing a recombinant nucleic acid molecule according to claim 3.
- 55 7. A method for producing a hog cholera virus polypeptide which polypeptide corresponds to a portion of the hog cholera virus precursor polypeptide, capable of inducing virus neutralising antibodies, wherein a host cell according to claim 4 or 5, or a recombinant virus according to claim 6 is propagated in a culture under conditions whereby the polypeptide is expressed, whereafter the polypeptide is isolated from the culture.

8. A vaccine for the protection of animals against hog cholera virus infection, characterised in that it comprises a hog cholera virus polypeptide which polypeptide corresponds to a portion of the hog cholera virus precursor polypeptide, capable of inducing virus neutralising antibodies, which polypeptide comprises the amino acid sequence located within the amino acid position 689-1067 shown in Fig 2 *in non-denatured and non-reduced form*, or is an antigenic fragment or functional equivalent thereof.
9. A vaccine for the protection of animals against hog cholera virus infection, characterised in that it comprises a hog cholera virus polypeptide *as obtainable by expression in a recombinant host cell* of a nucleic acid sequence corresponding to a fragment of the hog cholera virus genome, and encoding a hog cholera virus polypeptide or an antigenic fragment thereof, capable of inducing virus neutralising antibodies, which polypeptide comprises the amino acid sequence located within the amino acid position 689-1067 shown in Fig 2 or is a functional equivalent thereof.
10. A vaccine according to claim 9, wherein said nucleic acid sequence comprises either the nucleotide sequence 2428-3564 shown in Fig 2 or a fragment thereof, or a nucleotide sequence which hybridises under stringent conditions to said nucleotide sequence shown in Fig 2.
11. A vaccine for the protection of animals against hog cholera virus infection, characterised in that it comprises a host cell according to claims 4-5 or a recombinant virus according to claim 6.
12. A method for the preparation of a hog cholera virus vaccine, characterised in that a polypeptide as defined in claims 8-10 is formed to a pharmaceutical preparation with immunising activity.
13. A method for the preparation of a hog cholera vaccine, characterised in that a host cell according to claims 4-5 or a recombinant virus according to claim 6 is propagated in a culture, whereafter the host cell or the recombinant virus is harvested and is formed to a pharmaceutical preparation with immunising activity.

Claims for the following Contracting States : ES, GR

1. A process for the preparation of a recombinant nucleic acid molecule which comprises incorporating a nucleic acid sequence corresponding to a fragment of the hog cholera virus genome, and encoding a hog cholera virus polypeptide or an antigenic fragment thereof, capable of inducing virus neutralizing antibodies, which polypeptide comprises the amino acid sequence located within the amino acid position 689-1067 shown in figure 2 or is a functional equivalent thereof, in a vector nucleic acid molecule.
2. A process according to claim 1, characterized in that said nucleic acid sequence comprises, either the nucleotide sequence 2428-3564 shown in figure 2 or a fragment thereof, or a nucleotide sequence which hybridizes under stringent conditions to said nucleotide sequence shown in figure 2.
3. A process which comprises expressing in a host cell the nucleic acid sequence defined in claim 1 or 2.
4. A process according to claims 3, characterized in that the host cell is a bacterium.
5. A process for the preparation of a recombinant virus, characterized in that the nucleic acid sequence defined in claim 1 or 2 is incorporated into a virus.
6. A process according to claims 3 or 4 which comprises allowing expression of the nucleic acid sequence in a culture, and recovering the polypeptide from the culture.
7. A process for the preparation of a vaccine for the protection of animals against hog cholera virus infection comprising, following the process of claim 6, the forming of the polypeptide expression product to a pharmaceutical preparation with immunizing activity.
8. A process for the preparation of a vaccine for the protection of animals against hog cholera virus infection comprising forming a host cell capable of expressing the nucleic acid sequence defined in claims 1 or 2, or a recombinant virus comprising the nucleic acid sequence defined in claims 1 or 2, to a pharmaceutical preparation with immunizing activity.

Patentansprüche

Patentansprüche für folgend Vertragsstaaten: BE, CH, DE, DK, FR, GB, IT, LI, NL, SE

- 5 1. Nukleinsäuresequenz, die einem Fragment des Schweinepestvirusgenoms entspricht und ein Schweinepestvirus-Polypeptid oder antigenisches Fragment desselben codiert, das dazu fähig ist, virusneutralisierende Antikörper zu induzieren, wobei das Polypeptid die in der in Figur 2 gezeigten Aminosäureposition 689-1067 vorliegende Aminosäuresequenz umfaßt oder ein funktionelles Äquivalent davon ist.
- 10 2. Nukleinsäuresequenz gemäß Anspruch 1, dadurch gekennzeichnet, daß sie entweder die in Figur 2 dargestellte Nukleotidsequenz 2428-3564 oder ein Fragment derselben oder eine Nukleotidsequenz, die unter strengen Bedingungen mit der in Figur 2 dargestellten Nukleotidsequenz hybridisiert, enthält.
- 15 3. Rekombinantes Nukleinsäuremolekül mit einem Vektornukleinsäuremolekül und einer Nukleinsäuresequenz gemäß Anspruch 1 oder 2.
4. Wirtszelle, die dazu fähig ist, ein Schweinepestvirus-Polypeptid mit einem rekombinanten Nukleinsäuremolekül gemäß Anspruch 3 zu produzieren.
- 20 5. Wirtszelle gemäß Anspruch 4, dadurch gekennzeichnet, daß es sich dabei um ein Bakterium handelt.
6. Rekombinantes Virus mit einem rekombinanten Nukleinsäuremolekül gemäß Anspruch 3.
- 25 7. Verfahren zur Herstellung eines Schweinepestvirus-Polypeptids, das einem Teil des Schweinepestvirus-Vorläuferpolypeptids entspricht und dazu fähig ist, virusneutralisierende Antikörper zu induzieren, bei dem eine Wirtszelle gemäß Anspruch 4 oder 5 oder ein rekombinantes Virus gemäß Anspruch 6 in einer Kultur unter Bedingungen, unter denen das Polypeptid expremiert wird, gezüchtet wird, wonach man das Polypeptid aus der Kultur isoliert.
- 30 8. Impfstoff zum Schutz von Tieren gegen Infektion mit Schweinepestvirus, dadurch gekennzeichnet, daß er ein Schweinepestvirus-Polypeptid enthält, das einem Teil des Schweinepestvirus-Vorläuferpolypeptids entspricht und dazu fähig ist, virusneutralisierende Antikörper zu induzieren, wobei das Polypeptid die innerhalb der in Figur 2 dargestellten Aminosäureposition 689-1067 befindliche Aminosäuresequenz *in nichtdenaturierter und nichtreduzierter Form* enthält oder ein antigenisches Fragment oder funktionelles Äquivalent davon ist.
- 35 9. Impfstoff zum Schutz von Tieren gegen Infektion mit Schweinepestvirus, dadurch gekennzeichnet, daß er ein Schweinepestvirus-Polypeptid in der Form enthält, *wie es durch Expression in einer rekombinanten Wirtszelle einer Nukleinsäuresequenz erhalten werden kann*, die einem Fragment des Schweinepestvirus-Genoms entspricht und ein Schweinepestvirus-Polypeptid oder antigenes Fragment davon codiert, das fähig ist, virusneutralisierende Antikörper zu induzieren, wobei das Polypeptid die innerhalb der in Figur 2 dargestellten Aminosäureposition 689-1067 befindliche Aminosäuresequenz umfaßt oder ein funktionelles Äquivalent davon ist.
- 40 10. Impfstoff gemäß Anspruch 9, wobei die Nukleinsäuresequenz entweder die in Figur 2 dargestellte Nukleotidsequenz 2428-3564 bzw. ein Fragment davon oder eine Nukleotidsequenz, die unter strengen Bedingungen mit der in Figur 2 dargestellten Nukleotidsequenz hybridisiert, enthält.
- 45 11. Impfstoff zum Schutz von Tieren gegen Infektion mit Schweinepestvirus, dadurch gekennzeichnet, daß er eine Wirtszelle gemäß Ansprüchen 4-5 oder ein rekombinantes Virus gemäß Anspruch 6 enthält.
- 50 12. Verfahren zur Herstellung eines Schweinepestvirus-Impfstoffs, dadurch gekennzeichnet, daß ein wie in den Ansprüchen 8-10 definiertes Polypeptid als pharmazeutische Zusammensetzung mit immunisierender Wirksamkeit formuliert wird.
- 55 13. Verfahren zur Herstellung eines Schweinepest-Impfstoffs, dadurch gekennzeichnet, daß eine Wirtszelle gemäß den Ansprüchen 4-5 oder ein rekombinantes Virus gemäß Anspruch 6 in einer Kultur gezüchtet wird, wonach die Wirtszelle bzw. das rekombinante Virus geemtet wird und als pharmazeutische Zusammensetzung mit immunisierender Wirksamkeit formuliert wird.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Ein Verfahren zur Herstellung eines rekombinanten Nukleinsäuremoleküls, welches die Einverleibung einer Nukleinsäuresequenz entsprechend einem Fragment des Schweinepestvirusgenoms und das Codieren eines Schweinepestviruspolypeptids oder eines antigenen Fragmentes davon, das fähig ist, virusneutralisierende Antikörper zu induzieren, wobei das Polypeptid die innerhalb der in Figur 2 dargestellten Aminosäurestellung 689-1067 liegende Aminosäuresequenz umfasst oder ein funktionelles Äquivalent davon ist, in ein Vektornukleinsäuremolekül umfasst.
2. Ein Verfahren nach Anspruch 1, dadurch gekennzeichnet, dass die genannte Nukleinsäuresequenz entweder die in Figur 2 dargestellte Nukleotid-Sequenz 2428-3564 oder ein Fragment davon oder eine Nukleotid-Sequenz, welche unter stringenteren Bedingungen zu der genannten, in Figur 2 dargestellten Nukleotidsequenz hybridisiert, umfasst.
3. Ein Verfahren, welches die Expression der in Anspruch 1 oder 2 definierten Nukleinsäuresequenz in eine Wirtszelle umfasst.
4. Ein Verfahren nach Anspruch 3, dadurch gekennzeichnet, dass die Wirtszelle ein Bakterium ist.
5. Ein Verfahren zur Herstellung eines rekombinanten Virus, dadurch gekennzeichnet, dass die in Anspruch 1 oder 2 definierte Nukleinsäuresequenz in einen Virus einverleibt wird.
6. Ein Verfahren nach Anspruch 3 oder 4, welches das Expressierenlassen der Nukleinsäuresequenz in eine Kultur und das Gewinnen des Polypeptids aus der Kultur umfasst.
7. Ein Verfahren zur Herstellung eines Impfstoffes zum Schutz von Tieren gegen Schweinepestvirusinfektion, umfassend, im Anschluss an das Verfahren nach Anspruch 6, die Verarbeitung des Polypeptid-Expressionsproduktes zu einem pharmazeutischen Präparat mit immunisierender Wirkung.
8. Ein Verfahren zur Herstellung eines Impfstoffes zum Schutz von Tieren gegen Schweinepestvirus-Infektion, umfassend die Verarbeitung einer Wirtszelle, die fähig ist, die in Anspruch 1 oder 2 definierte Nukleinsäuresequenz zu exprimieren, oder eines rekombinanten Virus, welcher die in Anspruch 1 oder 2 definierte Nukleinsäuresequenz umfasst, zu einem pharmazeutischen Präparat mit immunisierender Wirkung.

Revendications

Revendications pour les Etats contractants suivants : BE, CH, DE, DK, FR, GB, IT, LI, NL, SE

1. Séquence d'acide nucléique correspondant à un fragment du génome du virus de la peste porcine, et codant pour un polypeptide du virus de la peste porcine ou un fragment antigénique de celui-ci, susceptible d'induire des anticorps de neutralisation de virus, lequel polypeptide comprend la séquence d'acides aminés localisée au sein de la position des acides aminés 689-1067 représentée à la fig. 2 ou est un équivalent fonctionnel de celle-ci.
2. Séquence d'acide nucléique selon la revendication 1, caractérisée en ce qu'elle comprend soit la séquence nucléotidique 2428-3564 représentée à la fig. 2 ou un fragment de celle-ci, soit une séquence nucléotidique s'hybridant dans des conditions stringentes à ladite séquence nucléotidique représentée à la fig. 2.
3. Molécule d'acide nucléique recombinant comprenant une molécule d'acide nucléique vecteur et une séquence d'acide nucléique selon la revendication 1 ou la revendication 2.
4. Cellule hôte susceptible de produire un polypeptide du virus de la peste porcine comprenant une molécule d'acide nucléique recombinant selon la revendication 3.
5. Cellule hôte selon la revendication 4, caractérisée en ce que la cellule hôte est une bactérie.
6. Virus recombinant contenant une molécule d'acide nucléique recombinant selon la revendication 3.

7. Procédé de production d'un polypeptide du virus de la peste porcine, lequel polypeptide correspond à une portion de la polyprotéine du précurseur du virus de la peste porcine, susceptible d'induire des anticorps de neutralisation de virus, caractérisé en ce qu'une cellule hôte selon la revendication 4 ou 5, ou un virus recombinant selon la revendication 6 est propagé en culture dans des conditions telles que le polypeptide est exprimé, puis le polypeptide est isolé de la culture.
8. Vaccin destiné à protéger les animaux contre l'infection par le virus de la peste porcine, caractérisé en ce qu'il comprend un polypeptide du virus de la peste porcine, lequel polypeptide correspond à une portion de la polyprotéine précurseur du virus de la peste porcine, susceptible d'induire des anticorps de neutralisation de virus, lequel polypeptide comprend la séquence d'acides aminés localisée au sein de la position des acides aminés 689-1067 représentée à la fig. 2 sous forme non dénaturée et non réduite, ou est un fragment antigénique ou un équivalent fonctionnel de celle-ci.
9. Vaccin destiné à protéger les animaux contre l'infection par le virus de la peste porcine, caractérisé en ce qu'il comprend un polypeptide du virus de la peste porcine tel qu'on peut l'obtenir par l'expression dans une cellule hôte recombinante d'une séquence d'acide nucléique correspondant à un fragment du génome du virus de la peste porcine, et codant pour un polypeptide du virus de la peste porcine ou un fragment antigénique de celui-ci, susceptible d'induire des anticorps de neutralisation de virus, lequel polypeptide comprend la séquence d'acides aminés localisée au sein de la position des acides aminés 689-1067 représentée à la fig. 2 ou est un équivalent fonctionnel de celle-ci.
10. Vaccin selon la revendication 9, caractérisé en ce que ladite séquence d'acide nucléique comprend soit la séquence nucléotidique 2428-3564 représentée à la fig. 2 ou un fragment de celle-ci, soit une séquence nucléotidique s'hybridant dans des conditions stringentes à ladite séquence nucléotidique représentée à la fig. 2.
11. Vaccin destiné à protéger les animaux contre l'infection par le virus de la peste porcine, caractérisé en ce qu'il comprend une cellule hôte selon les revendications 4-5 ou un virus recombinant selon la revendication 6.
12. Procédé de préparation d'un vaccin du virus de la peste porcine, caractérisé en ce qu'un polypeptide tel que défini dans les revendications 8-10 est formé en une préparation pharmaceutique à activité immunisante.
13. Procédé de préparation d'un vaccin du virus de la peste porcine, caractérisé en ce que la cellule hôte selon les revendications 4-5 ou un virus recombinant selon la revendication 6 est propagé en culture, puis la cellule hôte ou le virus recombinant est récolté et formé en une préparation pharmaceutique à activité immunisante.

Revendications pour les Etats contractants suivants : ES, GR

1. Un procédé de préparation d'une molécule d'acide nucléique recombinante qui comprend l'incorporation d'une séquence nucléotidique correspondant à un fragment du génome du virus de la peste porcine, et codant pour un polypeptide du virus de la peste porcine ou un fragment antigénique de ce dernier, capable d'induire des anticorps neutralisants le virus, lequel polypeptide comprend la séquence d'acides aminés localisée entre les positions d'acide aminé 689-1067 décrite à la figure 2 ou correspond à un équivalent fonctionnel de ce dernier, dans une molécule d'acide nucléique vecteur.
2. Un procédé selon la revendication 1 caractérisé en ce que ladite séquence nucléotidique comprend soit la séquence nucléotidique 2428-3564 décrite à la figure 2 soit un fragment de cette dernière soit une séquence nucléotidique qui s'hybride dans des conditions drastiques à ladite séquence nucléotidique décrite à la figure 2.
3. Un procédé qui comprend l'expression dans une cellule hôte de la séquence d'acides nucléiques définie dans la revendication 1 ou 2.
4. Un procédé selon la revendication 3 caractérisée en ce que la cellule hôte est une bactérie.
5. Un procédé de préparation d'un virus recombinant caractérisé en ce que la séquence d'acides nucléiques définie dans la revendication 1 ou 2 est incorporée dans un virus.
6. Un procédé selon la revendication 3 ou 4 qui comprend l'expression de la séquence d'acides nucléiques dans une

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culture et la récupération du polypeptide à partir de la culture.

- 5 7. Un procédé de préparation d'un vaccin pour la protection des animaux contre une infection par le virus de la peste porcine comprenant, en suivant le procédé de la revendication 6, la transformation du produit d'expression du polypeptide en une préparation pharmaceutique présentant une activité immunisante.
- 10 8. Un procédé de préparation d'un vaccin pour la protection des animaux contre une infection par le virus de la peste porcine comprenant la transformation d'une cellule hôte capable d'exprimer la séquence d'acides nucléiques définie dans les revendications 1 ou 2 ou d'un virus recombinant comprenant la séquence d'acides nucléiques définie dans les revendications 1 ou 2, en une composition pharmaceutique présentant une activité immunisante.

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Figure 2.

1 GTT AGC TCT TTC TCG TAT ACC ATA TTG GAT ACA CTA AAT TTC GAT TTG GTC TAG GGC ACC CCT CCA GCG ACG GCC GAA ATG GGC 84
 85 TAG CCA TGC CCA TAG TAG GAC TAG CAA ACG GAG GGA CTA GCC GTA GTC GCG ACC TCC CTG GGT CTA AGT CCT GAG TAC ACG 168
 169 ACA GTC GTC AGT AGT TCG ACG TGA GCA CTA GCC CAC CTC GAG ATG CTA COT GGA CGA GGG CAT GCC CAA GAC ACA CCT TAA CCC 252
 253 TGG CGG GGG TCG CTA GGG TGA AAT CAC AAT ATG TGA TGG GGG TAC GAC CTG ATA GGG TGC TGC AGA GGC CCA CTA GCA GGC TAG 336
 337 TAT AAA AAT CTC TGC TGT ACA TGG CAC ATG GAG TTG AAT CAT TTT GAA TTA TAC AAA ACA AGC AAA CAA AAA CCA CTG GCA 420
 1 Met Glu Leu Asn His Phe Glu Leu Leu Tyr Lys Thr Ser Lys Glu Lys Pro Val Gly 19
 421 GTG GAG GAA CCG GTG TAT GAC ACC GCG GGG AGA CCA CTA TTT GGG AAC CCA AGT GAG GTA CAC CCA CAA TCA ACG CTG AAG CTG 504
 20 Val Glu Glu Pro Val Tyr Asp Thr Ala Gly Arg Pro Leu Phe Glu Asn Pro Ser Glu Val His Pro Glu Ser Thr Leu Lys Leu 47
 505 CCA CAC GAC ACG GGG AGA GGA GAT ATC AGA ACA ACA CTG ACG GAC CTA CCC AGG AAA GGT GAC TGT AGG AGT GGC AAC CAT CTA 588
 48 Pro His Asp Arg Gly Arg Gly Asp Ile Arg Thr Thr Leu Arg Asp Leu Pro Arg Lys Glu Asp Cys Arg Ser Gly Asn His Leu 75
 589 GGC CCG GTT AGT AGT GGG ATA TAC ATA AAG CCC GGC CCT GTC TAC TAT CAG GAC TAC ACG GGC CCA GTC TAT CAC AGA GCT CCT TTA 672
 76 Gly Pro Val Ser Gly Ile Tyr Ile Lys Pro Gly Pro Val Tyr Tyr Glu Asp Tyr Thr Gly Pro Val Tyr His Arg Ala Pro Leu 103
 673 GAG TTC TTT GAT GAG GGC CAG TTC TGC GAG GTC ACT AAG AGA ATA GGC AGG GTC ACG GGT AGT GAT GGT AAG CTT TAC CAC ATA 756
 104 Glu Phe Phe Asp Glu Ala Glu Phe Cys Glu Val Thr Lys Arg Ile Gly Arg Val Thr Gly Ser Asp Gly Lys Leu Tyr His Ile 131
 757 TAT GTC TGC GAT GAT GGT TGC ATA CTG ATA TTA GGC AAA AGG GGC ACA CCC AGA ACC CTA AAG TGG ATT AGG AAC TTC ACC 840
 132 Tyr Val Cys Val Asp Gly Cys Ile Leu Leu Lys Leu Ala Lys Arg Gly Thr Pro Arg Thr Leu Lys Trp Ile Arg Asn Phe Thr 159
 841 AAC TGT CCA TTA TGG GTA ACC AGT TGC TCC GAT GAC GGC GCA AGT GGC AGC AAG GAT AAG AAG CCA GAC AGA ATG AAC AAA GGT 924
 160 Asn Cys Pro Leu Trp Val Thr Ser Cys Ser Asp Asp Gly Ala Ser Gly Ser Lys Asp Lys Lys Pro Asp Arg Met Asn Lys Gly 187
 925 AAG TTG AAG ATA GCC CCA AGA GAG CAT GAG AAG GAC AGC AAG ACC AAG CCT CCT GAT GCA ACG ATT GTA GTA GAG GGA GTA AAA 1008
 188 Lys Leu Lys Ile Ala Pro Arg Glu His Glu Lys Asp Ser Lys Thr Lys Pro Pro Asp Ala Thr Ile Val Val Glu Gly Val Lys 215
 1009 TAC CAA ATC AAA AAG AAA GGC AAA GTC AAA GGG AAG ACA CAA GAC GGC CTG TAC CAT AAT AAG AAC AAG CCA CCA GAG TCC 1092
 216 Tyr Glu Ile Lys Lys Lys Gly Lys Val Lys Gly Lys Asn Thr Glu Asp Gly Leu Tyr His Asn Lys Asn Lys Pro Pro Glu Ser 263

1093 AGG AAG AAA CTA GAA AAA GCC CTG TTG GCT TGG GCG ATA ACA ATC TTG TAC CAG CCT GTA GCA GCC GAG AAC ATA ACT 1176
 214 Arg Lys Lys Leu Glu Lys Ala Leu Leu Ala Trp Ala Val Ile Thr Ile Leu Leu Tyr Glu Pro Val Ala Ala Glu Asn Ile Thr 271
 1177 CAA TGG AAC CTG AGT GAC AAC GGC ACT AAT GGT ATT CAG CGA GCC ATG TAT CTT AGA GGG GTT AAC AGG AGC TTA CAT GGG ATC 1260
 272 Glu Trp Asn Leu Ser Asp Asn Gly Thr Asn Gly Ile Glu Arg Ala Met Tyr Leu Arg Gly Val Asn Arg Ser Leu Met Glu Ile 299
 1261 TGG CCC GAG AAA ATA TGC AAG GGG GTC CCC ACT CAT CTG GCC ACT GAC AGC GAA CTG AAA CAG ATA CCG GGG ATG ATG GAT GCC 1344
 300 Trp Pro Glu Lys Ile Cys Lys Lys Gly Val Pro Thr Met Lys Leu Ala Thr Asp Thr Glu Leu Lys Glu Ile Arg Gly Met Met Asp Ala 327
 1345 AGC GAG AGG ACA AAC TAT ACG TGC TGT AGG TTA CAA AGA CAT GAA TGG AAC AAA CAT CGA TGG TGT AAC TGG TAC AAC ATA GAC 1428
 328 Ser Glu Arg Thr Asn Tyr Thr Cys Cys Arg Leu Glu Arg Met Glu Trp Asn Lys Met Gly Trp Cys Asn Trp Tyr Asn Ile Asp 355
 1429 CCT TGG ATT CAG TTA ATG AAC AGC ACC CAA ACA AAT TTG ACA GAA GGC CCT CCA GAT AAG GAG TGT GCC GTG ACC TGC AGG TAT 1512
 356 Pro Trp Ile Glu Leu Met Asn Arg Thr Glu Thr Asn Leu Thr Glu Gly Pro Pro Asp Lys Glu Cys Ala Val Thr Cys Arg Tyr 381
 1513 GAC AAA AAT ACC GAT GTC AAC GTC ACC GAG GTC ACC CAG GCC AGC AAT AGG CCA ACT ACT CTG ACT GGC TGC AAG AAA GGG AAA AAC TTT 1596
 304 Asp Lys Asn Thr Asp Val Asn Val Val Thr Glu Ala Ala Arg Asn Arg Pro Thr Thr Leu Thr Gly Cys Lys Lys Gly Lys Asn Phe 411
 1597 TCA TTC GCA GGC ACA GTC ATA GAG GGC CCG TGC AAT TTC AAC GTT TCC GTG GAG GAC ATC TTA TAC GCA GAC CAT GAG TGT GGC 1680
 412 Ser Phe Ala Gly Thr Val Ile Glu Gly Pro Cys Asn Phe Asn Val Ser Val Glu Asp Ile Leu Tyr Gly Asp Met Glu Cys Gly 439
 1681 AGT CTG CTC GAG GAC ACG GCT CTG TAC CTA TTG GAT GGA ATG ACC AAC ACT ATA GAG AAT GCC AAG CAA GGT GCG GCG GGTG 1764
 440 Ser Leu Leu Glu Asn Thr Ala Leu Tyr Leu Leu Asp Gly Met Thr Asn Thr Ile Glu Asn Ala Arg Glu Gly Ala Ala Arg Val 467
 1765 ACA TCT TGG CTT GGG AAG CAG CTC AGT ACC GCA GGG AAG AAG CTA GAG AGG AGA AGC AAA ACC TGG TTT GGT GCC TAT GCC CTG 1848
 468 Thr Ser Trp Leu Gly Arg Glu Leu Ser Thr Thr Ala Gly Lys Leu Glu Arg Arg Ser Lys Thr Trp Phe Gly Ala Tyr Ala Leu 495
 1849 TCA CCT TAC TGC AAT GTC ACT AGA AAA ATA GGG TAC ATA TGG TAT ACA AAC TGC ACC CCG GCA TGC CTC CCT AAG AAC ACA 1932
 496 Ser Pro Tyr Cys Asn Val Thr Arg Lys Ile Gly Tyr Ile Trp Tyr Thr Asn Asn Cys Thr Pro Ala Cys Leu Pro Lys Asn Thr 523
 1933 AAA ATA ATA GGC CCT GGA AAG TTT GAC ACC AAT GCG GAA GAC GGG AAG ATC CTT CAT GAA ARG GGG GCC CAC CTA TCA GAA TTT 2016
 524 Lys Ile Ile Gly Pro Gly Lys Phe Asp Thr Asn Ala Glu Asp Gly Lys Ile Leu Met Glu Met Gly Gly Met Leu Ser Glu Phe 551
 2017 TTG TTG CTT TCT CTA GTT ATC CTG TCT GAC TTT GCC CCC GAG ACA GCT AGC ACG CTA TAC CTA ATT TTA CAC TAT GCA ATC CCC 2100
 552 Leu Leu Leu Ser Leu Val Ile Leu Ser Asp Phe Ala Pro Glu Thr Ala Ser Thr Leu Tyr Leu Ile Leu Met Tyr Ala Ile Pro 579

218101	CAG TCC CAC GAA GAA CCT GAT GGT TGT GAT ACG AAC CAA CTT AAC CTA ACA GTG AAA CTT AGG ACA GAA GAC GTA GTG CCA TCA	2181
580	Gln Ser His Glu Glu Pro Glu Gly Cys Asp Thr Asn Gln Leu Asn Leu Thr Val Lys Leu Arg Thr Glu Asp Val Val Pro Ser	607
218185	TCA GGT TGG AAT ATT GGC AAA TAT GTT TGT GTT AGA CCA GAC TGG TGG CCG TAT GAA ACT AAA GTG GCT CTG CTO TTT GAA GAG	2186
608	Ser Val Trp Asn Ile Gly Lys Tyr Val Cys Val Arg Pro Asp Thr Tyr Glu Thr Lys Val Ala Leu Leu Phe Glu Glu	635
218189	GCA GGA CAG GTT ATA AAG CTA GTC CTA CCG GCA CTG AGG GAT TTA ACT AGG GTC TGG AAC AGC GCA TCA ACT ACT GCG TTT CTC	2192
616	Ala Gly Gln Val Ile Lys Leu Val Leu Arg Ala Leu Arg Asp Leu Thr Arg Val Trp Asn Ser Ala Ser Thr Thr Ala Phe Leu	663
218193	ATT TGC TTG ATA AAA GTA TTG AGA GGA CAG GGT GTG CAA GGT ATA ATA TGG CTG CTG GCG GCA CAA GCG CCG CTA	2196
664	Ile Cys Leu Ile Lys Val Leu Arg Gly Gln Val Val Gln Gly Ile Ile Trp Leu Leu Val Thr Gly Ala Gln Gly Arg Leu	691
218197	GCC TGT AAG GAA GAC TAC AGG TAT GCG ATC TCG TCA ACC AAT GAG ATA GGG CTG GCG GCT GAA GGT CTC ACC ACT ACC TGG	2200
692	Ala Cys Lys Glu Asp Tyr Arg Tyr Ala Ile Ser Ser Thr Asn Glu Ile Gly Leu Leu Gly Ala Glu Gly Leu Thr Thr Trp	719
218201	AAA GAA TAC AGC CAC GGT TTG CAG CTG GAC GGA ACC GTT AAG GCC GTC TGC ACT GCA GGG TCC TTT AAA GTC ACA GCA CTT	2604
720	Lys Glu Tyr Ser His Gly Leu Gln Leu Asn Asp Gly Thr Val Lys Ala Val Cys Thr Ala Gly Ser Phe Lys Val Thr Ala Leu	747
218205	AAC GTG GTT AGT AGG AGG TAT CTA GCA TCA TTG CAC AAG AGG GCT CTA CCC ACC TCA GTC GTC ACA TTT GAG CTC CTA TTT GAC GGG	2608
728	Asn Val Val Ser Arg Arg Tyr Leu Ala Ser Leu His Lys Arg Ala Leu Pro Thr Ser Val Thr Phe Glu Leu Phe Asp Gly	775
218209	ACC AAC GCA GCA ATC CAG GAG ATG GAT GAT GAT TTC GGA TTT GGG CTG TGC CCA TTT GAC ACG AGT CCT GTG ATC AAA GGG AAG	2772
776	Thr Asn Pro Ala Ile Glu Glu Met Asp Asp Phe Gly Phe Gly Leu Cys Pro Phe Asp Thr Ser Pro Val Ile Lys Gly Lys	803
218273	TAC AAC ACC ACT TTG TTA AAC GGC AGT GCT TTC TAT CTA GTC TGC CCA ATA GGA TGG ACT GGT GTC GTA GAG TGC ACA GCA GTG	2856
804	Tyr Asn Thr Thr Leu Leu Asn Gly Ser Ala Phe Tyr Leu Val Cys Pro Ile Gly Trp Thr Gly Val Val Glu Cys Thr Ala Val	831
218287	AGC CCC ACA ACC TTG AGA ACA GAA GTG GTG AAA ACC TTC AGG AGA GAT AAG CCT TTT CCA CAT AGA GTA GAC TGT GTG ACC ACC	2940
812	Ser Pro Thr Thr Leu Arg Thr Glu Val Val Lys Thr Phe Arg Arg Asp Lys Pro Phe Pro His Arg Val Asp Cys Val Thr Thr	859
218291	ATA GTA GAA AAA GAA GAC CTA TTC CAT TGC AAG TTG GGG GGT AAT TGG ACA TGT GTA AAA GCC GAC CCA GTC ACT TAT AAG GCG	3024
860	Ile Val Glu Lys Glu Asp Leu Phe His Cys Lys Leu Gly dly Asn Trp Thr Cys Val Lys Gly Asp Pro Val Thr Tyr Lys Gly	887
218295	GGG CAA GTA AAG CAG TGC AGG TGG TGT GGT TTC GAG TTT AAA GAG CCC TAC GGG CTC CCA CAC TAC CCT ATA GGC AAG TGC ATC	3108
888	Gly Gln Val Lys Gln Cys Arg Trp Cys Gly Phe Glu Phe Lys Glu Pro Tyr Gly Leu Pro His Tyr Pro Ile Gly Lys Cys Ile	915

3109 CTA ACA AAT GAG ACA GGT TAC AGG GTA GTA GAT TCC ACA GAC TGC AAC AGA GAT GGC CTC GTT ATT AGC ACT GAA GCG GAA CAT 3192
 916 Leu Thr Asn Glu Thr Gly Tyr Arg Val Val Asp Ser Thr Asp Cys Asn Arg Asp Gly Val Val Ile Ser Thr Glu Gly Glu His 943

 3193 GAG TGC TTG ATT GGC AAC ACT ACC GTC AAG GTC CAT GCA CTG GAT GAA AGA TTG GGC CCT ATG CCG TGC AGA CCC AAA GAA ATC 3276
 944 Glu Cys Leu Ile Gly Asn Thr Thr Val Val Lys Val His Ala Leu Asp Glu Arg Leu Gly Pro Met Pro Cys Arg Pro Lys Glu Ile 971

 3277 GTC TCT AGT AGT GAG GGA CCT GTG AGG AAA ACT TCT TGT ACA TTC AAC TAC ACA AAG ACT CTA AGA AAC AAA TAC TAT GAG CCC AGA 3360
 972 Val Ser Ser Glu Gly Pro Val Arg Lys Thr Ser Cys Thr Phe Asn Tyr Thr Lys Thr Leu Arg Asn Lys Tyr Ty- Glu Pro Arg 999

 3361 GAC AGT TAC TTC CAG CAA TAT ATG CTC AAG GGC GAG TAT CAA TAC TGG TTT AAT CTG GAC GTG ACC GAC CAC CAC ACA GAC TAC 3444
 1000 Asp Ser Tyr Phe Glu Glu Phe Met Leu Lys Gly Glu Tyr Glu Tyr Trp Phe Asn Leu Asp Val Thr Asp His His Thr Asp Tyr 1027

 3445 TTT GCC GAG TTT GTT GTC TTG GTA GTA GCA CTG TTA GGA GGA AGG TAC GGT CTG TCG CTA ATA GTG ACC TAC TAC ATA ATT CTA 3528
 1028 Phe Ala Glu Phe Val 1055

 3529 ACA GAG CAG CTC GCT GCT CTA CAG CTA GGC CAG GGT GAG GTG GTA TTG ATA GGG AAC CTA ATT ACC CAC ACG GAC AAT GAG 3612
 1056 Thr Glu Glu Leu Ala Ala Gly Leu Glu Leu Glu Gly Glu Val Val Val Val Val Val Val Val Val Val Val Val Val Val Val Val 1083

 3613 GTG GTC TAC TTC CTA CTC TAC TTA GTA ATA AGA GAT GAG CCC ATA AAG AAA TGG ATA CTA CTG CTG TTT CAT GCA ATG 3696
 1084 Val Val Val Tyr Phe Leu Leu Leu Tyr Leu Val Ile Arg Asp Glu Pro Ile Lys Lys Trp Ile Leu Leu Leu Phe His Ala Met 1111

 3697 ACT AAC AAT CCA GTC AAC ACC ATA ACA GTA GCA TTG CTA ATG ATC AGT GGG GTT GCC AAG GGT GGT AAG ATA GAT GGT GGC TGG 3780
 1112 Thr Asn Asn Pro Val Lys Thr Ile Thr Val Ala Leu Leu Met Ile Ser Gly Val Ala Lys Gly Lys Ile Asp Gly Gly Trp 1139

 3781 CAG AGA CAA CCG GTG ACC AGT TTT GAC ATC CAA CTC GCA CTG GCA GTC GTA GTC GTT GTG ATG TTG CTG GCA AAG AGA GAC 3864
 1140 Glu Arg Glu Pro Val Thr Ser Phe Asp Ile Glu Leu Ala Leu Ala Val Val Val Val Val Val Val Val Val Val Val Val Val Val Val 1167

 3865 CCG ACT ACT TTC CCT TTG GTA ATC ACA GTG GCA ACC CTG AGA ACG GCC AAG ATA ACC AAC GGT TTT AGC ACA GAT CTA GTC ATA 3948
 1168 Pro Thr Thr Phe Pro Lys Val Ile Thr Val Ala Thr Leu Arg Thr Ala Lys Ile Thr Asn Gly Phe Ser Thr Asp Leu Val Ile 1195

 3949 GCC ACA GTG TCG GCA GCT TTG TTA ACT TGG ACC TAT ATC AGC GAC TAC AAA TAC AAG ACT TGG CTA CAG TAC CTC GTC AGC 4032
 1196 Ala Thr Val Ser Ala Ala Leu Leu Thr Trp Thr Tyr Ile Ser Asp Tyr Tyr Lys Thr Trp Leu Glu Tyr Leu Val Ser 1223

 4033 ACG GTG ACT GGA ATC TTC CTG ATA AGC GTG CTG AAG GGA ATA GGC GAA TTG GAT CTG CAC GCC CCA ACC TTG CCG TCT CAC AGA 4116
 1224 Thr Val Thr Gly Ile Phe Leu Ile Arg Val Leu Lys Gly Ile Gly Glu Leu Asp Leu His Ala Pro Thr Thr Leu Pro Ser His Arg 1251

4117 CCC CTC TTT TAC ATC CTT GTA TAC CTT ATT TCC ACT GCC GTG GTA ACT AGA TGG AAT CTG GAC GTA GCC GGA TTG TTG CTC CAG 4204
 1252 Pro Leu Phe Tyr Ile Leu Val Tyr Leu Ile Ser Thr Ala Val Val Thr Arg Trp Asn Leu Asp Val Ala Gly Leu Leu Leu Gln 1279

 4201 TGC GTC CCA ACT CTT TTA ATG GTT TTT ACG ATG TGC GCA GAC ATT CTC ACC CTA ATT CTC ATA CTA CCT ACT TAT TAT GAG TTA ACA 4284
 1280 Cys Val Pro Thr Leu Leu Met Val Phe Thr Met Trp Ala Asp Ile Leu Thr Leu Ile Leu Pro Thr Tyr Glu Leu Thr 1307

 4285 AAG TTA TAC TAC CTT AAG GAA CTC AAG ATT GGG GCA GAA AOA GGT TGG CTA AAA ACT AAC TAT AAG AAG GTA AAC GAC ATC 4368
 1308 Lys Leu Tyr Tyr Leu Lys Glu Val Lys Ile Gly Ala Gly Arg Gly Trp Leu Trp Lys Thr Asn Tyr Lys Arg Val Asn Asp Ile 1335

 4369 TAC GAG GTC GAC CAA ACT AGC GAA GGG GTT TAC CTT TTC CCT TCT AAA CAG AAG ACG GCT ATA ACT AGT ACC ATG TTG CCA 4452
 1336 Tyr Glu Val Asp Gln Thr Ser Glu Gly Val Tyr Leu Phe Pro Ser Lys Gln Arg Thr Ser Ala Ile Thr Ser Thr Met Leu Pro 1363

 4453 TTA ATC AAA GCC ATA CTC ATT AGC TGC ATC AGC AAC AAG TGG CAA CTC ATA TAC TTA CTG TTT ATA TTT GAA GTG TCT TAC 4536
 1364 Leu Ile Lys Ala Ile Leu Ile Ser Cys Ile Ser Asn Lys Trp Gln Leu Ile Tyr Leu Leu Tyr Leu Ile Phe Glu Val Ser Tyr 1391

 4537 TAC CTC CAC AAG AAA GTT ATA GAT GAA ATA GCT GGT GGG ACC AAC TTC TCA AAG CTC GGT GCG GCT TTT ATT GAA GTC AAT 4620
 1392 Tyr Leu His Lys Lys Val Ile Asp Glu Ile Ala Gly Ile Ala Gly Thr Asn Phe Val Ser Arg Leu Val Ala Ala Leu Ile Glu Val Asn 1619

 4621 TGG GCC TTC GAC AAT GAA GTC AAA GGC TTA AAG AAG TTC TTC TTG CTG TCT AGT AGG GTC AAA GAG TTG ATC ATC AAA CAC 4704
 1420 Trp Ala Phe Asp Asn Asn Glu Glu Val Lys Gly Leu Lys Lys Phe Leu Leu Ser Ser Arg Val Lys Glu Leu Ile Ile Lys His 1667

 4705 AAA GTG AAG AAT GAA GTA GTG GTC CGC TGG TTT GGA GAT GAA GAG ATT TAT GGG ATG CCA AAG CTG ATC GGC TTA GTT AAG GCA 4788
 1468 Lys Val Arg Asn Glu Val Val Val Arg Trp Phe Gly Asp Glu Ile Tyr Gly Met Pro Lys Leu Ile Gly Leu Val Lys Ala 1675

 4789 GCA ACA GTA AGT AGA AAC AAA CAC TGT ATG TTG TGT ACC GTC TGT GAG GAC AGA CAT TGG AGA GGG GAA ACT TGC CCT AAA TGT 4872
 1476 Ala Thr Leu Ser Arg Asn Lys His Cys Met Leu Cys Thr Val Cys Glu Asp Arg Asp Trp Arg Gly Glu Thr Cys Pro Lys Cys 1503

 4873 GCG CGT TTT GGA CCA CCA GTC GTC TGC GGT ATG ACC CTA GCC GAT TTC GAA GAA CAC TAT AAA AAG ATT TTC ATT AGA GAG 4954
 1504 Gly Arg Phe Gly Pro Pro Val Val Cys Gly Met Thr Leu Ala Asp Phe Glu Lys His Tyr Lys Arg Ile Phe Ile Arg Glu 1535

 4957 GAC CAA TCA GGC GGG CCA CTT AAG GAG GAG CAT GCA GGG TAC TGG CAG TAC AAA GCC AGG GGT CAA CTG TTT TTG AAG AAC CTC 5040
 1532 Asp Gln Ser Gly Gly Pro Leu Leu Arg Glu Glu His Ala Gly Tyr Leu Gln Tyr Lys Ala Arg Gly Gln Leu Phe Leu Arg Asn Leu 1559

 5041 CCA GTG TTA GCT ACA AAA GTC AAG ATG CTC CTG GGT AAC CTC GGG ACA GAG ATT GGC GAT CTG GAA CAC CTT GGC TGG GTG 5124
 1560 Pro Val Leu Ala Thr Lys Val Lys Met Leu Leu Val Gly Asn Leu Gly Thr Glu Ile Gly Asp Leu Glu His Leu Gly Trp Val 1567

5125 CTT AGA GGG CCA GCT GTT TGC AAG AAG GTT ACT GAA CAC GAA AGA TGC ACC ACC TCT ATA ATG GAT AAG TTG ACT GCT TTC TTT 5308
 1588 Leu Arg Gly Pro Ala Val Cys Lys Lys Val Thr Glu His Glu Arg Cys Thr Thr Ser Ile Met Asp Lys Leu Thr Ala Phe Phe 1615

 5209 GGA GTA ATG CCA AGG GGC ACT ACT CCC AGA GCT CCC GTA AGA TTC CCT ACC TCC CTC CTA AAG ATA AGA AGA GGG CTC GAG ACT 5292
 1616 Gly Val Met Pro Arg Gly Thr Thr Pro Arg Ala Pro Val Arg Phe Pro Thr Ser Leu Leu Lys Ile Arg Arg Gly Leu Glu Thr 1643

 5293 GGT TGG GCT TAC ACA CAC CAA GGT GGC ATC GGC TCA GTA GAC CAT GTC ACT TGT GGG AAA GAC TTA CTG GTG TGT GAC ACC ATG 5376
 1644 Gly Trp Ala Tyr Thr His Glu Gly Ile Ser Ser Val Asp His Val Thr Cys Gly Lys Asp Leu Leu Val Cys Asp Thr Met 1671

 5377 GGT CGG ACA AGG GTT GTT TGC CAG TCA AAT AAT AAG ATG ACC GAG TCC GAA TAC GGA GTC AAA ACT GAC TCC GGG TGC CCA 5460
 1672 Gly Arg Thr Arg Val Val Cys Glu Ser Asn Asn Lys Met Thr Asp Glu Ser Glu Tyr Gly Val Lys Thr Asp Ser Gly Cys Pro 1699

 5461 GAG GGA GGC AAG TGT TAC GTG TTT AAC CCG GAA GCA GCT AAC ATA TCA GGC ACT AAA GGA GGC ATG GTC CAC TTA CAG AAA ACG 5544
 1708 Glu Gly Ala Arg Cys Tyr Val Phe Asn Pro Glu Ala Val Asn Ile Ser Gly Thr Lys Gly Ala Met Val His Leu Glu Lys Thr 1727

 5545 GGT GGA GAA TTC ACC TGT GTG ACA GCA TCA GGA ACC CCG GCT TTC TTT GAC CTC AAG AAC CTT AAG GGC TGG TCA GGG CTA CCG 5628
 1728 Gly Gly Glu Phe Thr Cys Val Thr Ala Ser Gly Thr Pro Ala Phe Phe Asp Leu Lys Asn Leu Lys Gly Trp Ser Gly Leu Pro 1755

 5629 ATA TTT GAA GCA TCA AGT GGA AAG GTA GTC GGA AGG GTC AAG GTC GGG AAG AAC GAG GAT TCC AAA CCA ACC AAG CTC ATG AGT 5712
 1756 Ile Phe Glu Ala Ser Ser Gly Arg Val Val Gly Arg Val Lys Val Gly Lys Asn Glu Asp Ser Lys Pro Thr Lys Leu Met Ser 1783

 5713 GGG ATA CAA ACG GTT TCT AAA AAG GCC ACA GAC TTA ACG GTG AAG AAG ATA ACG ACC ATG AAC AAG GGA GAG TTC AGA 5796
 1784 Gly Ile Glu Thr Val Ser Lys Ser Ala Thr Asp Leu Thr Glu Met Val Lys Lys Ile Thr Thr Met Asn Arg Gly Glu Phe Arg 1811

 5797 CAA ATA ACC CTG GCC ACA GGT GCC GGA AAA ACT ACA GAG CTC CCT AGA TCA GTT ATA GAA GAG ATA GGG AAG CAT AAG AGG GTG 5880
 1812 Glu Ile Thr Leu Ala Thr Gly Ala Gly Lys Thr Thr Glu Leu Pro Arg Ser Val Ile Glu Glu Ile Gly Arg His Lys Arg Val 1839

 5881 TTG GTC TTA ATC CCC TTA AAG GCG GCA GCA GCA TCA GTA TAC CAA TAC ATG AGA CAG AAA CAT CCG AGT ATA GCA TTC AAT CTA 5964
 1840 Leu Val Leu His Pro Leu Arg Ala Ala Ala Glu Ser Val Tyr Glu Tyr Met Arg Glu Lys His Pro Ser Ile Ala Phe Asn Leu 1867

 5965 AAG ATA GGT GAG ATG AAG GAA GGT GAT ATG GCT ACG GGA ATA ACC TAT GCT TCT TAC GGT TAC TTT TGC CAG ATG TCA CAA CCC 6048
 1868 Arg Ile Gly Glu Met Lys Glu Gly Asp Met Ala Thr Gly Ile Thr Tyr Ala Ser Tyr Gly Tyr Phe Cys Glu Met Ser Glu Pro 1893

 6049 AAG CTG AGA GCG GCA ATG GTA GAA TAT TCC TTT ATA TTC CTA GAT GAG TAT CAT TGT GCT ACC CCA GAA CAA CTG GCA ATC ATG 6132
 1896 Lys Leu Arg Ala Ala Met Val Glu Tyr Ser Phe Ile Phe Leu Asp Glu Tyr His Cys Ala Thr Pro Glu Glu Leu Ala Ile Met 1923

6111 GGG AAG ATC CAC AGA TTC TCA GAA AAC CTG GGG GTA GCT ATG ACA GCG ACA CCG GCA GGC ACA GTA ACA ACC ACT GCG CAG 6216
 1924 Gly Lys Ile His Arg Phe Ser Glu Asn Leu Arg Val Val Ala Met Thr Ala Thr Phe Ala Gly Thr Val Thr Thr Thr Gly Gln 1931

 6217 AAA CAC CCT ATA GAG GAA TTT ATA GCG CCG GAA GTC ATG AAA GGA GAC TTG GGT TCT GAG TAC TTA GAT ATT GCG GGA CTG 6300
 1932 Lys His Pro Ile Glu Glu Phe Ile Ala Pro Glu Val Met Lys Lys Gly Glu Asn Leu Gly Ser Glu Tyr Leu Asp Ile Ala Gly Leu 1939

 6301 AAG ATA CCA GTA GAG GAG ATG AAG AAT AAC ATG CTA GTT TTT GTG CCC ACC AGG AAC ATG GCG GTA GAG GCG GCA AAG AAA TTC 6384
 1980 Lys Ile Pro Val Glu Glu Met Lys Asn Asn Met Leu Val Phe Val Phe Thr Arg Asn Met Ala Val Glu Ala Ala Lys Lys Leu 2007

 6385 AAG GCG AAA GGA TAC AAC TCG GGC TAC TAC AGC GGA GAG CCA TCT AAC CTG AGG GTG GTG ACG TCG ACG TCG CAG TCC CCA TAC 6468
 2086 Lys Ala Lys Gly Tyr Asn Ser Gly Tyr Tyr Ser Gly Glu Asp Pro Ser Asn Leu Arg Val Val Thr Ser Ser Gln Ser Phe Tyr 2035

 6469 GTG GTG GTA GCA ACC AAC GCA ATA GAA TCG GGC GTT ACC CTC CCG GAC CTG GAC GTG GTT GTC GAC ACG GGA CTC AAG TGT GAA 6552
 2036 Val Val Val Ala Thr Asn Ala Ile Glu Ser Gly Val Thr Leu Pro Asp Leu Asp Val Val Val Asp Thr Gly Leu Lys Cys Glu 2063

 6553 AAA AGA ATC CGA CTG TCA CCC AAG ATG CCT TTC ATA GTG ACG GGC CTG AAA AGA ATG GCG GTC ACT ATT GGG GAA CAA GCC CAG 6636
 2064 Lys Arg Ile Arg Leu Ser Pro Lys Met Pro Phe Ile Val Thr Gly Leu Lys Arg Met Ala Val Thr Ile Gly Glu Gln Ala Gln 2091

 6637 AGA AGA GCG AGG GTF GCA AGA GTC AAG CCC GGG AGA TAC TAC AAG AGT CAA GAA ACA CCT GTC GGC TCT AAA GAC TAC CAT TAT 6720
 2092 Arg Arg Gly Arg Val Gly Arg Val Lys Pro Gly Arg Tyr Tyr Arg Ser Gln Glu Thr Phe Val Gly Ser Lys Asp Tyr His Tyr 2119

 6721 GAC TTA TTG CAA GCC CAG AAG TAC GGC ATA GAA GAT GGG ATA AAT ATC ACC AAA TCC TTC AGA GAG ATG AAC TAC GAC TGG ACC 6804
 2120 Asp Leu Leu Gln Ala Gln Arg Tyr Gly Ile Glu Asp Gly Ile Asn Ile Thr Lys Ser Phe Arg Glu Met Asn Tyr Asp Trp Ser 2147

 6805 CTT TAT GAG GAA GAT AGC CTG ATG ATC ACA CAA CTG GAA ATC CTC AAC CAG TTG ATA TCA GAA GAG CTG CCG ATG GCA GTA 6888
 2148 Leu Tyr Glu Glu Asp Ser Leu Met Ile Thr Glu Leu Glu Ile Leu Asn Asn Leu Leu Ile Ser Glu Glu Leu Pro Met Ala Val 2175

 6889 AAA AAT ATA ATG GCG ACG ACC GAC CAC CCA GAA CCA ATT CAA CTC GCG TAT AAC AGC TAC GAG ACA CAG GTG CCG GTA TTA TTC 6972
 2176 Lys Asn Ile Met Ala Arg Thr Asp His Pro Glu Pro Ile Gln Leu Ala Tyr Asn Ser Tyr Glu Thr Gln Val Pro Val Leu Phe 2203

 6973 CCA AAA ATA AGA AAT GGA GAG GTG ACT GAT ACT TAC GAT AAT TAC ACC TTC CTC AAT GCA AGA AAA TTG GGA GAT GAC GTA CCC 7056
 2204 Pro Lys Ile Arg Asn Gly Glu Val Thr Asp Thr Tyr Asn Asn Tyr Thr Phe Leu Asn Ala Arg Lys Leu Gly Asp Asp Val Pro 2231

 7057 CCC TAC GTG TAT GCT ACA GAG GAT GAG TTG GCA CTG GAA CTG TTG GGC CTA GAT TGG CCG GAC CCA GGA AAC CAA GGC ACC 7140
 2232 Pro Tyr Val Tyr Ala Thr Glu Asp Glu Asp Leu Ala Val Glu Leu Leu Gly Leu Asp Trp Pro Asp Phe Gly Asn Gln Gly Thr 2259

7141 GTG GAA GCT GGC AGA GCA CTA AAA CAG GTG GTT GGT CTA ACA GCA GAG AAC GCC CTG CTA GTC GCC CTG GCT GTC GGC TAC GTG 7224
 2260 Val Glu Ala Gly Arg Ala Leu Lys Gln Val Val Gly Leu Ser Thr Ala Glu Asn Ala Leu Leu Val Ala Leu Phe Gly Tyr Val 2287

 7225 GCG TAC CAG GCG CTT TCA AAG AGA CAT ATA CCA GTG GTC ACA GAT ATA TAT TCA GTA GAA GAT CAC AAG CTA GAG GAC ACT ACG 7308
 2288 Gly Tyr Gln Ala Leu Ser Lys Arg His Ile Pro Val Val Thr Asp Ile Tyr Ser Val Glu Asp His Arg Leu Glu Asp Thr Thr 2315

 7309 CAC CTA CAG TAT GCT CCG AAT GCC ATC AAG ACG GAG GCG AAG GAA ACT GAA TTG AAG GAG CTG GCT CAG GCG GAT GTG CAG AGA 7392
 2316 His Leu Gln Tyr Ala Pro Asn Ala Ile Lys Thr Glu Gly Lys Glu Thr Glu Lys Glu Leu Ala Gln Glu Asp Val Glu Arg 2343

 7393 TGT GTG GAA GCA GTG ACC AAT TAT GCG AGA GAG GGC ATC CAA TTC ATG AAG TCG CAG GCA CTG AAA GTG AGA GAA ACC CCT ACC 7476
 2344 Cys Val Glu Ala Val Thr Asn Tyr Ala Arg Glu Gly Ile Gln Phe Met Lys Ser Gln Ala Leu Lys Val Arg Glu Thr Pro Thr 2371

 7477 TAT AAA GAG ACA ATG AAC ACC GTG GCA GAT TAT GTG AAA AAG TTT ATT GAG GCA CTG ACG GAT AGC AAG GAA GAC ATC ATT AAA 7560
 2372 Tyr Lys Glu Thr Met Asn Thr Val Val Ala Asp Tyr Val Lys Lys Phe Ile Glu Ala Leu Thr Asp Ser Lys Glu Asp Ile Ile Lys 2399

 7561 TAT GCG CTG TGG GCG GCA CAT ACG GCA TTG TAT AAG AGC ATT GGT GCC AGG CTT GGT CAC GAA ACC GCG TTC GCA ACT CTA GTT 7644
 2400 Tyr Gly Leu Trp Gly Ala His Thr Ala Leu Tyr Lys Ser Ile Gly Ala Arg Leu Gly His Glu Thr Ala Phe Ala Thr Leu Val 2427

 7645 GTG AAG TGG TTG GCA TTT GCG GCG GAG TCA ATA TCA GAC CAC ATA AAG CAA GCG GCC ACA GAC TTG GTC GTC GTC GCA ACT CTA GCG 7728
 2428 Val Lys Trp Leu Ala Phe Gly Gly Glu Ser Ile Ser Asp His Ile Lys Gln Ala Ala Thr Asp Leu Val Val Tyr Tyr Ile Ile 2455

 7729 AAC AGA CCT CAA TTC CCA GAA GAC ACA GAA ACA CAA GAA GCG AGA AAA TTT GTT GCC AGC CTG CTA GTC TCA GCT CTA GCG 7812
 2456 Asn Arg Pro Gln Phe Pro Gly Asp Thr Glu Thr Gln Gln Glu Gly Arg Lys Phe Val Ala Ser Leu Leu Val Ser Ala Leu Ala 2483

 7813 ACT TAT ACA TAC AAG AGC TGG AAC TAC AAT AAT CTG TCC AAA ATA GTT GAA CCG GCT TTG GCT ACC CTG CCC TAT GCC GCT AAA 7896
 2484 Thr Tyr Thr Tyr Lys Ser Trp Asn Tyr Asn Asn Leu Ser Lys Ile Val Glu Pro Ala Leu Ala Thr Leu Pro Tyr Tyr Ala Ala Lys 2511

 7897 GCC CTC AAG CTA TTT GCT CCT ACC CGA CTG GAG AGC GTT GTC ATA CTG AGC ACT GCA ATC TAC AAA ACA TAC CTA TCA ATA ACG 7980
 2512 Ala Leu Lys Leu Phe Ala Pro Thr Arg Leu Gln Ser Val Val Ile Leu, Ser Thr Ala Ile Tyr Lys Thr Tyr Leu Ser Ile Arg 2530

 7981 CGA GGC AAA AGT GAT GAT GGT CTA GGT ACA GGG GTT AGC GCG ACC ATG GAA ATT ATG TCA CAA AAC CCA GTA TCT GTG GGT ATA 8064
 2540 Arg Gly Lys Ser Asp Gly Leu Leu Gly Thr Gly Val Val Ser Ala Ala Met Glu Ile Met Ser Gln Asn Pro Val Ser Val Gly Ile 2567

 8065 GCA GTT ATG CTA GGG GGT GCT GTA GCA GCC CAC AAT GCA ATT GAA GCC AGT GAG CAA AAA AGA ACA CTA CTT ATG AAA GTC 8148
 2568 Ala Val Met Leu Leu Gly Val Gly Ala Val Ala His Asn Ala Ile Glu Ala Ser Glu Gln Lys Arg Thr Leu Leu Met Lys Val 2595

8149 TTT GTG AAA AAC TTC TTA GAC CAG GGC GGC ACC GAC GAA CTA GTC AAA GAG AGC CCT GAG AAA ATA ATA ATG GCT TTG TTC GAA
 2596 Phe Val Lys Asn Phe Leu Asp Gln Ala Ala Thr Asp Gln Val Lys Glu Ser Pro Gln Lys Ile Ile Met Ala Leu Phe Glu 26.

8233 GCG GTG CAA ACG GTG GGC AAC CCT CTT AGA TTA GTG TAC CAC CTC TAT GGA GTT TTC TAT AAA GGG TGG GAA GCA AAA GAG TTG
 2624 Ala Val Gln Thr Val Gly Asn Pro Leu Arg Leu Val Tyr His Leu Tyr Gly Val Phe Tyr Lys Gly Trp Gln Ala Lys Glu Leu 8316

8317 GCC CAA AGA ACA GGC AGC AAC CTT TTC ACC TTG ATA ATG TTC GAG GCT GTG GAA CTA CTG GGA GTA GAC AGT GAG GGA AAA
 2632 Ala Gln Arg Thr Ala Gly Thr Ala Arg Asn Leu Phe Thr Leu Ile Met Phe Gln Ala Val Gln Leu Leu Gly Val Asp Ser Gln Gly Lys 8400

8401 ATT CCG CAG CTA TCG AGC AAT TAC ATA CTA GAG CTC TTG TAT AAG TTC CCG GAC AAT ATC AAG TCT AGT AGG GAG ATA GCA
 2680 Ile Arg Gln Leu Ser Ser Asn Tyr Ile Leu Gln Leu Leu Tyr Lys Phe Arg Asp Asn Ile Lys Ser Ser Val Arg Gln Ile Ala 2679

8485 ATC ACG TGG GCC CCC TTT AGT TGC GAT TGG ACA CCA ACA GAT GAC AGA ATA GGG CTT CCC CAT GAC AAT TAC CTC CGA
 2708 Ile Ser Trp Ala Pro Ala Pro Phe Ser Cys Asp Trp Thr Pro Thr Asp Asp Arg Ile Gly Leu Pro His Asp Asn Tyr Leu Arg 8484

8569 GTG GAG ACA AAG TGC CCC TGT GGT TAC AGG ATG AAA GCG GTA AAA AAC TGC GCT GGG GAG TTG AGA CTT CTG GAG GAA GCG GGT
 2736 Val Gln Thr Lys Cys Pro Cys Gly Tyr Arg Met Lys Ala Val Lys Asn Cys Ala Gly Gln Leu Arg Leu Leu Gln Gly Gly 2707

8633 TCA TTC CTC TGC AGA AAT AAA TTC GGT AGA GGC TCA CAA AAC TAC AAG GTG ACA AAA TAC TAT GAT GAC AAT TTA TCA GAA ATA
 2764 Ser Phe Leu Cys Arg Asn Lys Phe Gly Arg Gly Ser Gln Asn Tyr Arg Val Thr Lys Tyr Tyr Asp Asn Asn Leu Ser Gln Ile 8736

8737 AAA CCA GTG ATA AGA ATG GAA CCA CAC GTG GAA CTG TAT TAC AAG GGG GCC ACT ATC AAA CTG GAT TTT AAC AAC AGT AAA ACG
 2792 Lys Pro Val Ile Arg Met Gln Gly His Val Gln Leu Tyr Tyr Lys Gly Ala Thr Ile Lys Leu Asp Phe Asn Asn Ser Lys Thr 2791

8821 GTA CTG GCA ACT GAC AAA TGG GAG GTT GAC CAC CTC ACC CTG GTT AAG GCA CTC AAG AGG TAC ACA GGG GCT GGA TAT CGA GCG
 2820 Val Leu Ala Thr Asp Lys Trp Gln Val Val Asp His Ser Thr Leu Val Arg Ala Leu Lys Arg Tyr Thr Gly Ala Gly Tyr Arg Gly 8820

8903 GCG TAT TGG GGT GAG AAA CCT AAC CAT AAA CAT CTG ATA CAG AGA GAC TGT GCA ACG ATT ACC AAA GAC AAG GTC TGC TTC ATC
 2868 Ala Tyr Leu Gly Gln Lys Pro Asn His Lys His Leu Ile Gln Arg Asp Cys Ala Thr Ile Thr Lys Asp Lys Val Cys Phe Ile 2847

8989 AAA ATG AAG AGA GGG TGT GCG TTC ACT TAT GAC CTA TCC CTC CAC AAC CTT ACC CCG CTA ATC GAA TTG GTA CAC AAG AAT AAC
 2876 Lys Met Lys Arg Gly Cys Ala Phe Thr Tyr Asp Leu Ser Leu His Asn Leu Thr Arg Leu Ile Gln Leu Val His Lys Asn Asn 8988

9073 CTG GAA GAT AQA ATC CCT GCT GTG ACG GTT ACA ACC TGG CTG GCC TAC ACA TTT GTG AAT GAA GAC ATA GCG ACC ATA AAA
 2904 Leu Gln Asp Arg Gln Ile Pro Ala Val Thr Val Thr Trp Leu Ala Tyr Thr Phe Val Asn Gln Asp Ile Gly Thr Ile Lys 2903

9157 CCA ACT TTT GGG GAA AAG GTG ACA CCG GAG GAG GAG GTA GTC TTG CAG CCT GCT GTG GAG ACA ACA CAT GTA
 2932 Pro Thr Phe Gln Lys Val Thr Pro Gln Lys Gln Gln Val Val Leu Gln Pro Ala Val Val Asp Thr Thr Asp Val 9156

9240 CCA ACT TTT GGG GAA AAG GTG ACA CCG GAG GAG GAG GTA GTC TTG CAG CCT GCT GTG GAG ACA ACA CAT GTA
 2959 Pro Thr Phe Gln Lys Val Thr Pro Gln Lys Gln Gln Val Val Leu Gln Pro Ala Val Val Asp Thr Thr Asp Val 2931

9241 GCC GTG ACC GTG GTA GGG GAA ACC TCT ACT ATG ACT ACA GGG GAG ACC CCG ACA ACA TTT ACC AGC TTA GGT TCG GAC TCG AAG 9124
 2960 Ala Val Thr Val Val Gly Glu Thr Ser Thr Thr Met Thr Thr Thr Pro Thr Phe Thr Ser Leu Gly Ser Asp Ser Lys 2987

 9325 GTC CGA CAA GTC CTG AAG CTG GGC GTG GAC GAT GGT CAA TAC CCC GGG CCT AAT CAG CAG AGA GCA AGC CTG CTC GAA GGT ATA 9408
 2988 Val Arg Glu Val Leu Lys Leu Gly Val Asp Asp Gly Glu Tyr Pro Gly Pro Asn Glu Ala Ser Leu Leu Glu Ala Ile 3015

 9409 CAA GGT GTG GAT GAA AGG CCC TCG GTA CTG ATA CTG GGG TCT GAT AAG GGC ACC TCC AAT AGG GTC AAG ACC GCA AAG AAT GTG 9492
 3016 Glu Gly Val Asp Glu Arg Pro Ser Val Leu Ile Leu Gly Ser Asp Lys Ala Thr Ser Asn Arg Val Lys Thr Ala Lys Asn Val 3043

 9493 AAG ATA TAT AGC AGC AGC GAC CCC CTG GAA CTG AGA GAA ATG ATG AAA AGG GGA AAA ATC CTA GTC GTA GCC TTG TCT AGA CTC 9576
 3044 Lys Ile Tyr Arg Ser Arg Asp Pro Leu Glu Leu Arg Glu Met Lys Arg Gly Lys Ile Leu Val Val Ala Leu Ser Arg Val 3071

 9577 GAT ACC GCT CTG AAA TTC GTT GAT TAC AAA GGC ACC TTC CTG ACC AGA GAG ACC CTA GAG GCA TTA AGT CTG GGT AAG CCT 9640
 3072 Asp Thr Ala Leu Leu Lys Phe Val Asp Tyr Lys Gly Thr Phe Leu Thr Arg Glu Thr Leu Glu Ala Leu Ser Leu Gly Lys Pro 3099

 9661 AAG AAA AGA GAC ATA ACT AAA GCA GAA GCA GAA TGG CTG CCG CTC GAA GAC CAA ATA GAA GAG CTG CCT GAC TGG TTC GCA 9744
 3100 Lys Lys Arg Asp Ile Thr Lys Ala Glu Ala Glu Trp Leu Leu Arg Leu Glu Asp Glu Ile Glu Leu Pro Asp Trp Phe Ala 3127

 9745 GCC AAG GAA CCC ATA TTT CTA GAA GGC AAC ATT AAA CGT GAC AAG TAT CAC CTG GTA GGG GAC ATA GGC ACT ATT AAA GAA AAA 9828
 3126 Ala Lys Glu Pro Ile Phe Leu Glu Ala Asn Ile Lys Arg Asp Lys Tyr His Leu Val Gly Asp Ile Ala Thr Ile Lys Glu Lys 3155

 9829 GCC AAA CAA CTG GGG GCA ACA GAC TCC ACA AAG ATA TCA AAG GAG GTT GGC GCG AAA GTG TAT TCT ATG AAG CTG AGT AAC TGG 9912
 3156 Ala Lys Glu Leu Gly Ala Thr Asp Ser Thr Lys Ile Ser Lys Glu Val Gly Ala Lys Val Tyr Ser Met Lys Leu Ser Asn Trp 3183

 9913 GTG ATA CAA GAA GAG AAT AAA CAA GGC AGC GCT GGC CCC CTG TTT GAA GAG CTC CTG CAA CAG TGC CCA CCC GGG GGC CAG AAC 9996
 3184 Val Ile Glu Glu Asn Lys Glu Gly Ser Leu Ala Pro Leu Phe Glu Leu Leu Glu Cys Pro Pro Gly Gly Glu Asn 3211

 9997 AAA ACC ACA CAT ATG GTC TCA GCC TAC CAA GCT CAA GGT CAA GGG AAT TGG GTG CCA GTT AGT TGC CAC GTG TTC ATG GGG ACC ATA 10080
 3212 Lys Thr Thr His Met Val Ser Ala Tyr Glu Leu Ala Glu Gly Asn Trp Val Pro Val Ser Cys His Val Phe Met Gly Thr Ile 3239

 10081 CCC GCC AGA AGA ACC AAG ACT CAT CCT TAT GAG GCA TAC GTT AAG CTA AGG GAG TTG GTA GAT GAA CAT AAG ATG AAG GCA TTA 10160
 3260 Pro Ala Arg Arg Thr Lys Thr His Pro Tyr Glu Ala Tyr Val Lys Leu Arg Glu Leu Val Asp Glu His Lys Met Lys Ala Leu 3267

 10165 TGT GGC GGA TCA AGT AAG CAC AAC GAA TGG GTA ATT GGC AAG GTC AAG TAT CAA GGA AAC CTG AGG ACC AAA CAC ATG 10248
 3268 Cys Gly Gly Ser Gly Leu Ser Lys His Asn Glu Trp Val Ile Gly Lys Val Lys Tyr Glu Gly Asn Leu Arg Thr Lys His Met 3295

10219 TTG AAC CCC GGA AAG GTG GCG GAG CAA CTG CAC AGA GAA GGG TAC AGC CAC AAT GTG TAT AAT AAG ACA ATA GGT TCA GTG ATG 10312
 3286 Leu Asn Pro Gly Lys Val Ala Glu Gln Leu His Arg Gly Tyr Arg His Asn Val Tyr Asn Lys Thr Ile Gly Ser Val Met 3323

 10333 ACA GCA ACT GGT ATC AGG CTG GAG AAG TTA CCT GTG GTT AGG GCC CAA ACA GAC ACA ACC AAC TTC CAC CAA GCA ATA AGG GAT 10416
 3324 Thr Ala Thr Gly Ile Arg Leu Glu Lys Leu Pro Val Val Arg Ala Gln Thr Asp Thr Asn Phe His Gln Ala Ile Arg Asp 3351

 10417 AAA ATA GAC AAG GAG GAG AAC CTA CAG ACC CCT GGC TTG CAT AAG AAG TTA ATG GAA GTC TTC AAT GCA TTA AAA AGA CCC GAG 10500
 3352 Lys Ile Asp Lys Glu Gln Asn Leu Gln Thr Pro Gly Leu His Lys Lys Leu Met Gln Val Phe Asn Ala Leu Lys Arg Pro Gln 3379

 10501 CTT GAG GCC TCT TAT GAC GCT GTC GAT TGG GAG GAA TTG GAG AGA GGA ATA AAT AGG AAG GGT GCT GCT GGT TTC TTC GAA CGC 10584
 3380 Leu Gln Ala Ser Tyr Asp Ala Val Asp Trp Glu Gln Leu Glu Arg Gly Ile Asn Arg Lys Gly Ala Ala Gly Phe Phe Gln Arg 3407

 10585 AAG AAC ATA GGA GAG GTT TTG GAT TCG GAA AAA AAT AAA GTC GAA GAG GTT ATT GAC AGT TTG AAA AAA GGT AGC AAT ATC AGA 10668
 3408 Lys Asn Ile Gly Gln Val Leu Asp Ser Gln Lys Asn Lys Val Gln Val Ile Asp Ser Leu Lys Lys Gly Arg Asn Ile Arg 3435

 10669 TAC TAC GAA ACT GCA ATC CCG AAA AAC GAG AAG AGG GAT GTC AAT GAT GAC TGG ACC GCT GGT GAT TTC GTA GAT GAG AAG AAG 10752
 3436 Tyr Tyr Glu Thr Ala Ile Pro Lys Asn Gln Lys Arg Asp Val Asn Asp Asp Trp Thr Ala Gly Asp Phe Val Asp Gln Lys Lys 3463

 10753 CCA AGA GTG ATA CAA TAC CCT GAG GCT AAA ACT AGG TTG GCT ATT ACT AAG GTA ATG TAC AAG TGG GTC AAA CAG AAG CCA GTT 10836
 3464 Pro Arg Val Ile Gln Tyr Pro Gln Ala Lys Thr Arg Leu Ala Ile Thr Lys Val Met Tyr Lys Trp Val Lys Gln Lys Pro Val 3491

 10837 GTC ATA CCG GGT TAT GAA GGT AAG ACA CCC CTG TTT CAA ATT TTT GAC AAA GTG AAG AAA GAA TGG GAT CAA TTC CAA AAC CCT 10920
 3492 Val Ile Pro Gly Tyr Gln Gly Lys Thr Pro Leu Phe Gln Ile Phe Asp Lys Val Lys Gln Trp Asp Gln Phe Gln Asn Pro 3519

 10921 GTG GCA GTT AGC TTT GAT ACC AAA GCG TGG GAT ACC ACA AGG GAT TTG GAG CTA ATA AGG GAT ATA CAG AAG TTC 11004
 3520 Val Ala Val Ser Phe Asp Thr Lys Ala Trp Asp Thr Gln Val Thr Thr Arg Asp Leu Gln Leu Ile Arg Asp Ile Gln Lys Phe 3547

 11005 TAT TTT AAA AAG AAA TGG CAC AAA TTC ATT GAC ACC CTA ACC AAG CAC ATG TCA GAA GTA CCC GTA ATC AAT GCC GAC GGG GAG 11060
 3506 Tyr Phe Lys Lys Lys Trp His Lys Phe Ile Asp Thr Leu Thr Lys His Met Ser Gln Val Pro Val Ile Ser Ala Asp Gly Gln 3575

 11089 GTA TAC ATA AGG AAA GGT CAG AGA GGC AGT GGG CAA CCT GAC ACG GCA GGC AAC AGC ATG TTG AAT GTG TTG ACA ATC GTG 11172
 3576 Val Tyr Ile Arg Lys Gly Gln Arg Gly Ser Gln Pro Asp Thr Ser Ala Gly Asn Ser Met Leu Asn Val Leu Thr Met Val 3603

 11173 TAT GCC TTC TGC GAG GCC ACG GGG GTA CCC TAC AAG AGT TTT GAC AGA GTG GCA AAG ATC CAT GTC TGC GCG GAT GAT GGT TTC 11235
 3604 Tyr Ala Phe Cys Glu Ala Thr Gly Val Pro Tyr Lys Ser Phe Asp Arg Val Ala Lys Ile His Val Cys Gly Asp Asp Gly Phe 3631

11257 CTG ATT ACC GAA AGA GCT CTC GGT GAG AAA TTT GCG AGT AAA GGA GTC CAG ATC CTA TAC GAA GCT GGG AAG CCT CAA AAG ATC 11140
 3632 Leu Ile Thr Gln Arg Ala Leu Gly Gln Lys Phe Ala Ser Lys Gln Val Gln Ile Leu Tyr Gln Ala Gly Lys Pro Gln Lys Ile 3639

 11311 ACT GAA GGG GAC AAG ATG AAA GTA GGC TAT CAG TTT GAT GAT ATC GAG TTC TCC CAT ACA CCA GTA CAA GTG AGG TGG TCA 11424
 3660 Thr Gln Gly Asp Lys Met Lys Val Ala Tyr Gln Phe Asp Asp Ile Gln Phe Cys Ser His Thr Pro Val Gln Val Arg Trp Ser 3687

 11425 GAC AAT ACT TCC AGC TAC ATG CCG GGA AGG AAC ACG ACT ACA ATC CTG GCT AAA ATG GCT ACA AGG TTG GAT TCC AGT GGT GAG 11508
 3688 Asp Asn Thr Ser Ser Tyr Met Pro Gly Met Pro Gly Asn Thr Thr Thr Ile Leu Ala Lys Met Ala Thr Arg Leu Asp Ser Ser Gly Gln 3715

 11508 AGG GGT ACT ATA GCA TAT GAG AAG GCA GTG GCG TTC AGC TTT TTG TTG ATG TAC TCC TCG AAC CCA CTG ATC AGA AGG ATA TGC 11592
 3716 Arg Gly Thr Ile Ala Tyr Gln Lys Ala Val Ala Phe Ser Phe Leu Leu Met Tyr Ser Trp Asn Pro Leu Ile Arg Arg Ile Cys 3743

 11593 TTA CTG GTG TTG TCA ACT GAG TTG CAA GTG AAG CCA GGG AAG TCA ACC ACC TAT TAC TAT GAA GGG GAC CCA ATA TCC GCT TAC 11676
 3744 Leu Leu Val Leu Ser Thr Gln Leu Leu Gln Val Arg Pro Gly Lys Ser Thr Thr Tyr Tyr Tyr Gln Gly Asp Pro Ile Ser Ala Tyr 3771

 11677 AAG GAA GTC ATT GGC CAC AAT CTC TTT GAC CTT AAA AGA ACA ACG TTC GAA AAG CTA GCA AAG TTA AAT CTC AGC ATG TCC ACG 11760
 3772 Lys Gln Val Ile Gly His Asn Leu Leu Phe Asp Leu Lys Arg Thr Ser Phe Gln Lys Leu Ala Lys Leu Asn Leu Ser Met Ser Thr 3799

 11761 CTC GGG GTG TGG ACT AGA CAC ACT AGC AAG AGA TTA CTA GAT TGT GTC AAT GTT GGC ACC AAA GAG GGC AAC TGG CTG GTC 11844
 3800 Leu Gly Val Trp Thr Arg His Thr Ser Lys Arg Leu Leu Gln Asp Cys Val Asn Val Gly Thr Lys Gln Gly Asn Trp Leu Val 3827

 11845 AAT GCA GAC AGA CTA GTG AGT AGT AAG ACA GGA AAG AAG TAT ATA CCT GGA GAG GGC CAC ACC CTA CAA GGG AAA CAT TAT GAA 11928
 3828 Asn Ala Asp Arg Leu Val Ser Ser Lys Thr Gly Asn Arg Tyr Ile Pro Gly Gln Gly His Thr Leu Gln Gly Lys His Tyr Gln 3855

 11929 GAA CTG ATA CTG GCA AGG AAA CCG ATC GGT AAC TTT GAA GGG ACC GAT AGG TAT AAC TTG GCG CCA ATA GTC AAT GTA GTG TTG 12012
 3856 Gln Leu Ile Leu Ala Arg Lys Pro Ile Gly Asn Phe Gln Gly Thr Asp Arg Tyr Asn Leu Gly Pro Ile Val Asn Val Val Leu 3883

 12013 AGG AGA CTA AAA ATT ATG ATG GGC CTG ATA GGA AGG GGG GTG TGA GCA TGG TTG TCG GAT GAT CCG GCC CTA TCA GTA GAA 12096
 3884 Arg Arg Leu Lys Ile Met Met Met Ala Leu Ile Gly Asn Gly Val End 3899

 12097 CCC TAT TGT AAA CAT TAA CTT ATT AAT TAT TTA GAT ACT ATT TAT TTA TTT TAT TAT TTA TTG AAT GAG CAA GTA CTG 12180

 12191 GTA CAA ACT ACC TCA TGT TAC CAC ACT ACA CTC ATT TTA ACA GCA CTT TAG CTG GAG GGA AAA CCC TGA COT CCA CAG TTG GAC 12264

 12265 TAA GGT AAT TTC CTA ACG GC 12284

Figure 3. Nucleotide sequence.

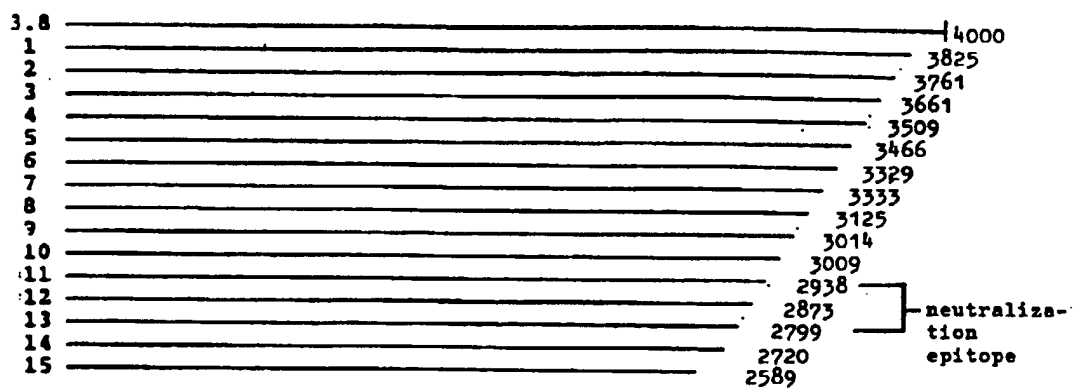
HCV

AGTGACAACGGCACTAATGGTATTCAGCGAGCCATGTATCTTAGAGGGGTAAACAGG
 AGCTTACATGGGATCTGGCCCGAGAAAATATGCAAGGGGGTCCCCACTCATCTGGCC
 ACTGACACGGAACTGAAAGAGATACGCGGGATGATGGATGCCAGCGAGAGGACAAAC
 TATACGTGCTGTAGGTTACAAAGACATGAATGGAACAAACATGGATGGTGTAAGTGG
 TACAACATAGACCCTTGGATTTCAGTTAATGAACAGGACCCAAACAAATTTGACAGAA
 GGCCCTCCAGATAAG

Deduced amino acid sequence.

HCV	S	D	N	G	T	N	G	I	Q	R	A	M	Y	L	R	G	V	N	R	S
	L	H	G	I	W	P	E	K	I	C	K	G	V	P	T	H	L	A	T	D
	T	E	L	K	E	I	R	G	M	M	D	A	S	E	R	T	N	Y	T	C
	C	R	L	Q	R	H	E	W	N	K	H	G	W	C	N	W	Y	N	I	D
	P	W	I	Q	L	M	N	R	T	Q	T	N	L	T	E	G	P	P	D	K

Figure 4



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(71) Applicant: **AKZO N.V.**
Velperweg 76
NL-6824 BM Arnhem(NL)

(72) Inventor: **Meyers, Gregor**
Gammertingerstrasse 79
D-7000 Stuttgart 80(DE)
Inventor: **Rümenapf, Tillmann**
Ligusterweg 3
D-7400 Tübingen(DE)
Inventor: **Thiel, Heinz-Jürgen**
Im Schönblick 67
D-7400 Tübingen(DE)

(74) Representative: **Hermans, Franciscus G.M. et al**
Patent Department AKZO N.V. Pharma
Division P.O. Box 20
NL-5340 BH Oss(NL)

(54) **Hog cholera virus vaccine and diagnostic.**

(57) The present invention is concerned with a hog cholera virus vaccine comprising a polypeptide characteristic of hog cholera virus. Vector vaccines capable to express a nucleic acid sequence encoding such a polypeptide also form part of the present invention. Said polypeptide and nucleic acid sequence can also be used for the detection of hog cholera virus infection.

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(73) Proprietor: **Akzo Nobel N.V.**
Velperweg 76
NL-6824 BM Arnhem (NL)

(72) Inventor: **Meyers, Gregor**
Gammertingerstrasse 79
D-7000 Stuttgart 80 (DE)
Inventor: **Rümenapf, Tillmann**
Ligusterweg 3
D-7400 Tübingen (DE)
Inventor: **Thiel, Heinz-Jürgen**
Im Schönblick 67
D-7400 Tübingen (DE)

(74) Representative: **Hermans, Franciscus G.M. et**
al
Patent Department
AKZO NOBEL N.V.
Pharma Division
P.O. Box 20
NL-5340 BH Oss (NL)

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